Evolutionary divergence of *Anguillicola crassus*, an invasive parasitic swim bladder nematode of eels of the genus *Anguilla*

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I dedicate this work to my son –

Without you I wouldn´t be as I am.

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Abstract

After its introduction from Taiwan to Europe at the beginning of the 1980s, *Anguillicola crassus*, a natural parasite of the Japanese eel (*Anguilla japonica*), had to face novel environmental conditions. As this jump dispersal of the nematode coincided with a host switch to the European eel (*Anguilla anguilla*), the parasite's novel host and new environment may have acted as substantially different selective forces leading to the onset of new phenotypic modifications and genetic adaptations of the parasite.

Here I conducted a common garden experiment under a reciprocal transplant design to investigate, if current populations of *A. crassus* from Germany, Poland and Taiwan differ in terms of recovery, developmental dynamics, reproductive potential and morphology while infecting the European and Japanese eels, and whether the differences are genetic or plastic responses to the new living conditions in Europe. Common garden studies estimate genetic adaptation by measuring fitness components under the same living conditions (in the European or Japanese eels), whereas the reciprocal transplant studies additionally incorporate the contribution of environmental variation comparing the parasite populations when harboured in different eel species.

My study revealed that the changes in infectivity (recovery), development, reproductive output and morphology observed in the parasite after colonization of the European eel were induced by both genetic adaptation and phenotypic plasticity. Under common garden conditions the parasite responded plastically with larger body and oesophagus dimensions, as well as higher reproductive output, to the different living conditions in the colonized area. In contrast, the recovery, developmental dynamics and morphology of oesophagus and buccal capsule have undergone a rapid evolutionary change (whereas the oesophagus simultaneously showed a high degree of phenotypic plasticity). In terms of infectivity and developmental dynamics, the current European populations showed a similar divergence pattern expressing lower recovery and faster development than their Taiwanese conspecifics. The morphological traits have diverged inhomogeneously for the both European populations: the Polish nematodes evolved a larger pharynx and buccal capsule than the German and Taiwanese populations and the German parasite had a smaller buccal capsule than the Taiwanese parasites. Genetically induced variability among the parasite strains in the reciprocally infected Japanese eel was less apparent. In conclusion, this study provides evidence of rapid genetic divergence of *A. crassus* at an ecological time scale after spatial isolation combined with a successful host switch.

Zusammenfassung

Anguillicola crassus ist ein Parasit des Japanischen Aals (*Anguilla japonica*), der Anfang der 1980er Jahre nach Europa eingeschleppt wurde. Neue Umweltbedingungen sowie der Wechsel zu einem neuen Wirt, dem Eurpäischen Aal (*Anguilla anguilla*), führten zu phenotypischen Modifikationen und möglicherwiese auch zu genetischen Veränderungen des Parasiten.

In meiner Arbeit führte ich Kreuzexperimente durch, in deren Verlauf Europäische und Japanische Aale mit *A. crassus* aus invasiven Populationen Deutschlands und Polens sowie aus dem natürlichen Verbreitungsgebiet in Taiwan infiziert wurden. Mit diesen Versuchen sollte geklärt werden, ob sich die drei Parasitenpopulationen in Bezug auf Infektiosität, Entwicklung, Reproduktionspotential und Morphologie unterscheiden. Diese Common Garden oder Transplantations-Versuche erlaubten darüber hinaus eine Unterscheidung zwischen genetisch fixierten Adaptionen und phänotypischer Plastizität aufgrund von Umweltbedingungen.

Meine Studie zeigte, dass den bei Europäischen Parasiten beobachteten Veränderungen der Infektiosität, Entwicklung, Reproduktionspotential und Morphologie sowohl genetische als auch umweltbedingte Faktoren zu Grunde liegen. Im neuen Wirt reagierten alle Parasiten-Stammen plastisch mit größerem Reproduktionspotential und einem größeren Körper und Oesophagus, sowie generell stärkerem Wachstum. Gleichzeitig konnte jedoch ein Teil der morphologischen Veränderungen sowie Variationen bei Wiederfindungsrate und Entwicklungszyklus auf eine ungewöhnlich schnelle Evolution zurückgeführt werden. Die europäischen Populationen von *A. crassus* zeigten bei schnellerer Entwicklung niedrigere Wiederfindungsraten als Würmer aus taiwanesischen Populationen. Die Unterschiede hinsichtlich morphologischer Merkmale waren jedoch nicht einheitlich für die beiden untersuchten europäischen Gebiete. Polnische Würmer zeigten eine vergrößerte Kopfkapsel und Pharynx im Vergleich zu allen anderen Populationen, wohingegen die Parasiten aus Deutschland eine kleinere Kopfkapsel aufwiesen als Tiere aus Taiwan. Im Gegensatz zu den Infektionsversuchen an Europäischen Aalen zeigten sich genetisch bedingte Unterschiede zwischen Europäischen und Taiwanesischen Würmern im Japanischen Aal weniger deutlich. Mit meiner Arbeit konnte ich somit deutliche Hinweise auf ungewöhnlich schnelle genetische Divergenz des invasiven Parasiten *A. crassus* als Folge von räumlicher Isolation und eines erfolgreichen Wechsels zu einem neuen Endwirt feststellen.

1 Introduction

1.1 Biological invasion

The transcontinental movement of people and commercial goods – a benchmark of the globalized world – is associated with the deliberate or unintended replacement of animals and plants. Alien, noxious, exotic, weedy, introduced, naturalized, transient, nuisance, nonindigenous, established and more - these terms are used alternately in the literature for the biological introductions. The number of species introduced to various environments is alarming. At least an estimated 480,000 have been cited (Colautti & MacIsaac, 2004) and they represent only that fraction of the total alien species, which could establish stable populations in the new areas (Williamson & Fitter, 1996). Nonindigenous invasive species (NIS) pose a multiple threat to global biodiversity as they endanger the natural homeostasis of the native fauna and flora (MacDougall & Turkington, 2005; El-Rashidy & Boxshall, 2009) on various trophic levels, often with disastrous ecological and economic consequences (Daszak et al., 2000; Pimentel et al., 2005; Strayer et al., 2006). For example, the estimated 50,000 NIS established in the United States cause environmental damage and economic losses of over US $125 billion per year (Pimentel et al., 2000).

Kolar and Lodge (2001) postulated three generalized steps in the invasion process: introduction, establishment and spread. According to Colautti and MacIsaac (2004), an invasion should run in five consecutive stages leading, through a series of biophysical filters, to the widespread and dominant occurrence of an introduced species in a target area. The dynamics of invasion is driven by the simultaneous interaction of ecological and evolutionary processes acting over parallel timescales (Lambrinos, 2004). In most cases an introduced species has to face novel physical, chemical and biological conditions such as climate, landscape patterns and community composition (Lonsdale, 1999; Mack et al., 2000) that often differ from the native area. The establishment and spread may start as a result of changes in external conditions in the colonized area, when species extend their range as a result, for example, of human mediated habitat change such as logging (Facon et al., 2006). The mosquito *Aedes aegypti*, the principal vector of dengue fever, evolved from forest relatives to be almost wholly commensal with humans driven by drastic human land-use changes (Monath, 1994). However, many introduced species may use preadaptations, maladaptations, or undergo an evolutionary change adapting to the new conditions (Facon et al., 2006). The adaptive changes can occur on morphological, behavioural or developmental levels (Mooney & Cleland, 2001; Reznick & Ghalambor, 2001). They are often observable in ecological time (Lee, 2002) and may result in divergence between the native and introduced

populations (Lambrinos, 2004). There is clear evidence from a range of taxa that introduced populations often adapt quickly to local conditions (Reznick & Ghalambor, 2001; Grosholz, 2002; Lee, 2002; Facon et al., 2006). For example the wing size in most species of *Drosophila* varies across latitudinal clines (Huey et al., 2000). *Drosophila subobscura* exhibited a clinal increase in body and female wing size after its introduction to the New World (Gilchrist et al., 2008). In the case of *Oryctolagus cuniculus* rabbits in Australia, the introduced population evolved changes in fecundity as a reaction to the new climate (Williams & Moore, 1989b). A wide array of various genetically based adaptations after colonization of the new hosts has been reported for herbivorous insects. *Acyrthosiphon* aphids evolved higher larval survival on the new host (Via et al., 2000), *Jadera* bugs exhibited changes in beak morphology, emergence time and performance on host (Carroll & Boyd, 1992; Carroll et al., 1997), while in *Prodoxus* moths changes in ovipositor size and shape as well as emergence time were observed (Groman & Pellmyr, 2000). *Euphydras* butterflies showed higher larval survival on a new host (Singer et al., 1993) and *Rhagoletis* fruit flies a change in diapause (Filchak et al., 2000). Interestingly, differences in diapause and mating behaviour have been shown between *Culex* mosquitoes living in the London underground railway system and surface dwelling populations (Byrne & Nichols, 1999).

An alternative way for many invasive species to deal with new environments is via phenotypic plasticity - the phenomenon of a genotype producing different phenotypes in response to different environmental conditions (Ghalambor et al., 2007). However, phenotypic plasticity and local adaptation may have a complementary influence on establishment and spread (Lambrinos, 2004), as shown for the salt cedar in North America (Sexton et al., 2002) and the Chinook salmon spreading to several drainage basins in the south island of New Zealand (Quinn et al., 2001). Phenotypic plasticity may be important in the early stages of invasions, allowing species to expand rapidly across diverse landscapes. Later, selection favours local adaptation that can lead to an increase in local invasiveness (Sexton et al., 2002; West-Eberhard, 2003).

Introduced species are often characterized by reduced genetic variation (bottle neck) due to only partial introduction of the total genetic variability from the source area (Roman & Darling, 2007). The reduced genetic variability could be also advantageous in the colonization process as was shown for Argentine ants. Due to the loss of a particular gene responsible for recognition of the queens by the workers and down-regulation of the population, these ants were able to set polygyne colonies, which had a negative impact on the native ant populations (Tsutsui & Suarez, 2003). The genetic variability can be additionally lowered by

random genetic drift, leaving even less variation for natural selection to work on. Propagule pressure, which includes both the number of introduced individuals and the number of release events, dynamically influences the progression of invasions and partly estimates the chances of success or failure of establishment in the new habitat (Lockwood et al., 2005). Both characteristics (the number of introduced individuals and the number of release events) are important for the genetic variability of the introduced population and have a special importance for invasive species that are limited by migration.

Among the NIS, parasites, which represent over 50% of the species of organisms extant today (Price, 1977; Bush, 2001), deserve special attention as they are often characterized by a higher virulence in their novel host compared to their native host (Carlquist, 1974; Clay, 2003). This increased virulence is presumably due to the lack of coadaptation processes between the new hosts and invasive parasites (Combes, 2001). There are several examples of dramatic consequences of parasitological introductions on naïve fish populations. In France multiple pathological effects on the colonized local fish fauna have been reported: the digenean *Bucephalus polymorphus* in small cyprinids (Wallet & Lambert, 1984) or the monogenean *Diplozoon nipponicum* and cestode *Bothriocephalus acheilognathi* in the common carp (Denis et al., 1983) are examples. Further, the introductions of *Gyrodactylus salaris* in the Atlantic salmon in Norway (Johnsen & Jenser, 1991), *Nitzschia sturionis* in the sturgeon in the Aral Sea (Zholdasova, 1997), the protozoan *Myxosoma cerebralis* in the rainbow trout in Europe (Hoffman, 1990) or *Pseudodactylogyrus* spp. and *Anguillicola crassus* in many populations of the European eel *Anguilla anguilla* in Europe (Koie, 1991) have substantially reduced their host population size in the invaded area. The latter species is the subject of this thesis.

1.2 *Anguillicola crassus* (Kuwahara, Niimi and Hagaki 1974)

1.2.1 Taxonomy

Anguillicola crassus is a parasitic, sangivorous nematode of eels of the genus *Anguilla*. It belongs, along with four other swim bladder parasites of fresh water eels, to the family Anguillicolidae (Figure 1). It was first described as *A. crassa* in specimens obtained from the Japanese eel, *Anguilla japonica* Temminck and Schlegel, 1847 (Kuwahara et al., 1974; Moravec & Taraschewski, 1988; Nagasawa et al., 1994; Evans & Matthews, 1999) based on morphology. Later, it was renamed *A. crassus* by Moravec and Taraschewski (1988). In 2006 Moravec transferred it into the genus *Anguillicoloides* (Moravec, 2006).

Phylum: Nematoda (Rudolphi, 1808)
Class: Secernentea (Linstow, 1905)
Order: Spirurida (Chitwood, 1933)
Superfamily: Anguillicoloidea (Yamaguti, 1935)
Family: Anguillicolidae (Yamaguti, 1935)
Genus: *Anguillicola* (Yamaguti, 1935)
A. globiceps (Yamaguti, 1935)
Genus: *Anguillicoloides* (Moravec et Taraschewski, 1988)
A. australiensis (Johnston et Mawson, 1940; Moravec et Taraschewski, 1988)
A. crassus (Kuwahara, Niimi et Itagaki, 1974; Moravec et Taraschewski, 1988)
A. novaezelandiae (Moravec et Taraschewski, 1988)
A. papernai (Moravec et Taraschewski, 1988)

Figure 1: Systematic classification of the Anguillicolidae (after Moravec (2006)).

In order to elucidate the phylogenetic affiliations within the Anguillicolidae and Nematoda, molecular approaches using the 18S rRNA gene of *A. crassus* were undertaken (Hirose et al., 1998; Wijová et al., 2006; Nadler et al., 2007) resulting, with the exclusion of the superfamily Dracunculoidea, in the erection of a new superfamily Anguillicoloidea. In a recent study, Lätsch (2010) verified methods for the identification of the nematode specimens using nuclear and mitochondrial DNA barcoding genes (18S rDNA, 28S rDNA D2-D3 and Cox1). The author questioned the division of the Anguillicolidae into the two genera *Anguillicola* and *Anguillicoloides*, therefore the former one is used in this thesis.

1.2.2 Geographical distribution

Until 1980 the geographical distribution of the Anguillicolidae was restricted to eels occurring in the Indo-pacific region (Moravec & Taraschewski, 1988) (Table 1). In the early 1980s the first reports on the occurrence of specimens of the genus *Anguillicola* in Europe appeared in the literature. Paggi et al. (1982) reported that 40% of eels in Lake di Bracciano in Italy were infected by *A. australiensis*. Later, several authors in Italy reported *A. australiensis* or *A. globiceps* (Saroglia et al., 1985; Sarti et al., 1985). Consequently, a debate concerning taxonomic affiliation of the nematodes took place (De Charleroy et al., 1987) until the genus *Anguillicola* was reviewed (Moravec & Taraschewski, 1988) and the alien found in Lake di

1. Introduction

Bracciano was classified as *A. novaezelandiae sp. nov*. Several years later all specimens found in Italy were identified as *A. crassus* (Moravec et al., 1994b; Münderle, 2005).

Table 1: Distribution of *Anguillicola* spp. and their final hosts, eels of the genus *Anguilla* (after Taraschewski (2006) and Sasal et al. (2008)).

***Anguillicola* ssp.**	**Native range**		**Colonized range**	
	Host species	Distribution	Host species	Distribution
A. crassus	*A. japonica* (Teminck & Schlegel,1847)	East Asia	*A. anguilla* (Linnaeus, 1758)	Europe
			A.rostrata (LeSueur,1821)	North America
			A. marmorata (Quoy & Gaimard, 1824)	East coast of South Africa
			A. mossambica (Peters, 1852)	East coast of South Africa
			A. bicolor bicolor (McClelland, 1844)	East coast of South Africa
A. novaezelandiae	*A. australis* (Richardson, 1841)	Australia and New Zealand	*A. anguilla* (Linnaeus, 1758)	Europe, Italy (no ability to spread)
	A. dieffenbachia ? (Gray, 1842)	New Zealand		
A. australiensis	*A. reinhardtii* (Steindachner, 1867)	South Australia		
A. papernai	*A. mossambica* (Peters, 1952)	East coast of South Africa		
A. globiceps	*A. japonica* (Teminck & Schlegel, 1847)	East Asia		

A. crassus gained special economic and biological status because of its excellent ability to become established and spread in new areas. Within three decades after its introduction to Europe, it spread over five novel eel species on three new continents: *Anguilla anguilla* in Europe and North Africa (Jakob et al., 2009b), *A. rostrata* in North and Central America (Johnson et al., 1995; Fries et al., 1996; Barse & Secor, 1999; Moser et al., 2000; Barse et al., 2001), *A. mossambica, A. marmorata* and *A. bicolor* on the east coast of South Africa

(Sasal et al., 2008) (Table 1; Figure 2). Recently, this nematode has been added to the list of the 100 worst exotic species in Europe (state on 12. 2011, http://www.europe-aliens.org/speciesTheWorst.do).

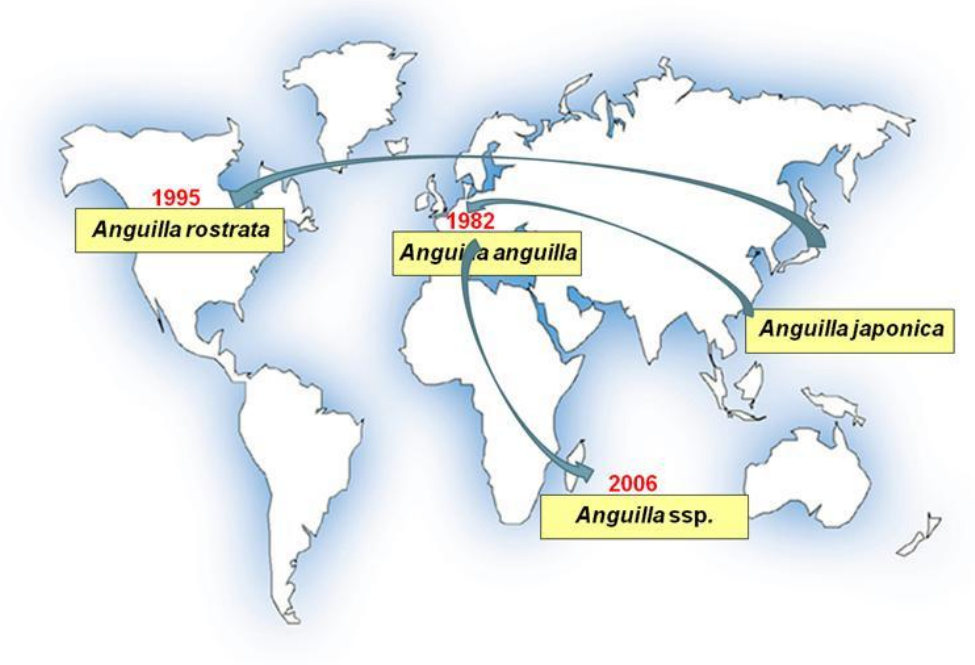

Figure 2: Expansion of *Anguillicola crassus* in Europe, America and Africa.

In its native range, *A. crassus* was reported from South Korea, Taiwan, Japan and China, in both, aquaculture and natural waters (Nagasawa et al., 1994; Moravec, 2006). From the available literature it can be concluded that *A. crassus* was introduced to Europe in the early 1980s with imported Japanese eels from Taiwan and New Zealand (Paggi et al., 1982; Neumann, 1985; Peters & Hartmann, 1986; Taraschewski et al., 1987; Belpaire et al., 1989b; Koops & Hartmann, 1989; Koie, 1991) or with re-exported European eels for consumption or restocking from Taiwan, Japan or China (Scholz, 1999). Wielgoss et al. (2008) using seven microsatellite loci and one mitochondrial marker, suggested that Europe was invaded via a single release event from Taiwan, whereas North America was colonized from Japan. The origin of the nematode in tropical eels remains unknown. It is possible that Africa was invaded secondarily via a European population source (Sasal et al., 2008).

There are several plausible explanations for the success of *A. crassus* as a global invader:

- it has invaded an empty niche in its new final hosts. The only other metazoan parasite found in the eel swim bladder in the invaded areas is another nematode, *Daniconema*

anguillae, but it mainly occupies the swim bladder wall and is rather rare (Moravec, 1994).

- it has a relatively simple life cycle (see chapter 1.2.3)
- it is able to use a wide range of common intermediate and paratenic hosts (Kennedy & Fitch, 1990; Szekely, 1994; Kirk, 2003).
- it has a high reproductive output (Knopf & Mahnke, 2004).

The process of establishment and spreading of the parasite in various parts of the world was consequently monitored and reported by parasitologists (Sasal et al., 2008; Aieta & Oliveira, 2009; Jakob et al., 2009b). Especially in Europe scientists have dealt very extensively with the dynamics of *A. crassus* invasion (Moravec, 1992; Kirk, 2003). The parasite was first observed in 1982 in European eels in northern Germany (Neumann, 1985). Within the following 16 years it was able to spread over almost all of the populations of the European eel (Kirk, 2003) (Figure 3; Table 2). Its colonization process was largely facilitated by anthropogenic transfers (Belpaire et al., 1989a; Koops & Hartmann, 1989; Kennedy & Fitch, 1990; Möller et al., 1991). In addition, natural movements of eels in fresh, brackish and coastal waters have accelerated the dispersion and extended the range of *A. crassus* throughout Europe (Kirk, 2003). Piscivorous birds, such as cormorants, might also play a role in extending of the dissemination of *A. crassus* via fish regurgitation (Wlasow et al., 1998). After its introduction to Europe *A. crassus* became the most abundant helminth of the European eel (Sures et al., 1999a). The establishment and spread of the parasite have proceeded similar in most European countries: the infection spread rapidly reaching the prevalences of up to 100% (Belpaire et al., 1989a; Kennedy & Fitch, 1990; Van Banning & Haenen, 1990; Székely et al., 1991; Thomas & Ollevier, 1992b; Własow et al., 1998; Palikova & Navratil, 2001; Reimer, 2002; Audenaert et al., 2003; Morozinska-Gogol, 2005) and mean intensities up to 66 worms per eel (Rolbiecki et al., 2000) with individuals harboring up to 356 worms (Höglund et al., 1992) in the first few years after introduction. Within one decade the rates of prevalence and mean intensity of infection have stabilized in almost all countries at around 50 – 90% and 3 - 7 worms per eel, respectively (Kennedy & Fitch, 1990; Haenen et al., 1994a; Molnár et al., 1994; Molnár & Székely, 1995; Würtz, 1998; Lefebvre et al., 2002b; Audenaert et al., 2003; Schabuss et al., 2005; Knopf, 2006).

Figure 3: First records of *Anguillicola crassus* in Europe (for references see Table 2).

Table 2: First records of *Anguillicola crassus* in European countries.

First record	Country	Reference
1982	Germany	Neumann, 1985
1985	Belgium	Belpaire et al., 1989a
1985	France	Dupont & Petter, 1988
1985	Holland	Van Banning et al., 1985
1986	Denmark	Koie, 1988
1987	Spain	Belpaire et al., 1989a
1987	England	Kennedy & Fitch, 1990
1987	Italy	Canestri-Trotti, 1987
1988	Sardinia (Italy)	Moravec, 1992
1988	Poland	Koops & Hartmann, 1989
1988	Estonia	Kangur, 1994
1988	Sweden	Hellstrom et al., 1988
1988	Greece	Belpaire et al., 1989a
1988?	Egypt	Koops & Hartmann, 1989

First record	Country	Reference
1988	Austria	Konecny & Wais, 1993
1990	Hungary	Székely et al., 1991
1991	Czech Republic	Moravec, 1992
1992	Belarus	After Moravec, 1992
1992	Yugoslavia	After Höglund & Thomas, 1992
1992	Portugal	Cruz et al., 1992
1993	Russia, Kaliningrad Oblast	Zaostrovceva, 1993
1993	Norway	Mo & Steien, 1994
1994	Latvia	Vismanis et al., 1999
1994	Tunisia	Maamouri et al., 1999
1994	Marocco	El Hilali et al., 1996
1995	Macedonia	Cakic et al., 2002
1998	Lithuania	Bacevicus, 2004
1998	Ireland	Evans & Matthews, 1999
1999	Algeria	After Nabil et al., 2009
2002	Corsica (France)	Ternengo et al., 2005
2002	Turkey	Genc et al., 2005
2004	Scotland	Lyndon & Pieters, 2005
2007	Finland	Jakob et al., 2009b

Parasite-dependent regulation of infrapopulation density (Ashworth & Kennedy, 1999; Fazio et al., 2008b), thickening of the swim bladder wall preventing re-infection with the nematode (Würtz and Taraschewski, 2000), mortality of the most heavily infected eels (Lefebvre et al., 2002a), and adaptations of the final host such as speeding up the silvering process (Fazio, 2007; Fazio et al., 2008a) were proposed as possible mechanisms keeping the infection intensity on a lower level than directly after introduction.

In comparison, in its native range the rates of prevalence and mean intensity are around 17.5 – 60% and 1.5 – 4.4 (range 1 - 20) worms per eel, respectively (Münderle et al., 2006; Han et al., 2008; Heitlinger et al., 2009).

1.2.3 The developmental cycle

A. crassus moults four times during its life and has a similar life cycle compared to other Anguillicolidae (Moravec and Taraschewski, 1988; Kirk, 2003) (Figure 4).

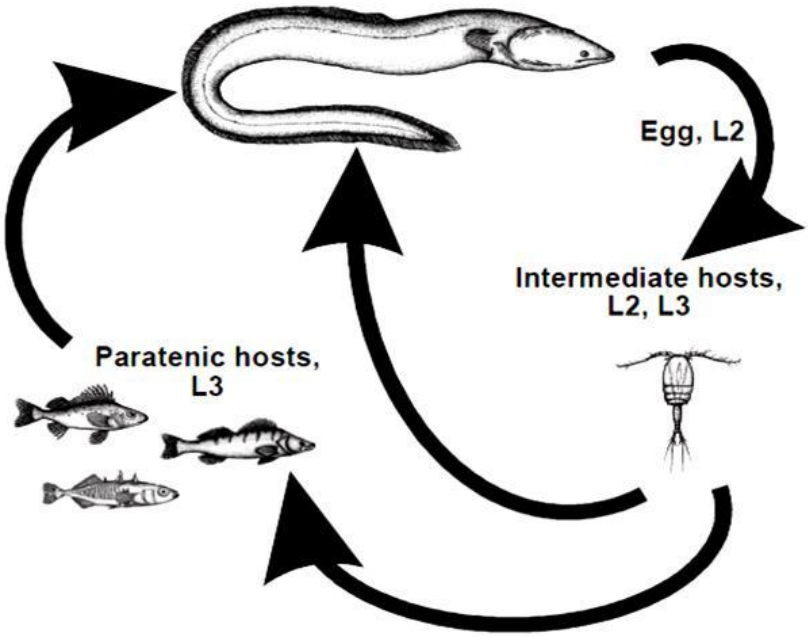

Figure 4: Developmental cycle of *Anguillicola crassus* (after Rolbecki and Rokicki (2005)).

Its indirect development requires an obligate intermediate and a final host (De Charleroy et al., 1990; Nagasawa et al., 1994). The only obligatory final host is an eel of the genus *Anguilla* that can be infected as early as the glass eel stage (Kennedy & Fitch, 1990; Nimeth et al., 2000). It has been shown that young eels with a length of 6 - 7 cm, which have just left their marine environment, can already be infected (Van Banning & Haenen, 1990). Copepods, ostracodes and malacostraca serve as obligatory intermediate hosts. Additionally a large number of paratenic hosts can be involved in spreading this parasite.

The division of the nematode into different larval stages was previously defined according to the data from wild eels (Hirose et al., 1976; Puqin & Yuru, 1980) and later verified by experimental observations (Haenen et al., 1989; De Charleroy et al., 1990). L2 larvae, which partly hatch inside of the swim bladder, are released through the pneumatic duct (De Charleroy et al., 1990) and then move via the digestive track (Puqin & Yuru, 1980) into the water (Kirk, 2000). In fresh water they hatch within few hours (De Charleroy et al., 1990; Kennedy & Fitch, 1990). After hatching they fasten themselves by their tail-end to the benthic substrate and wriggle their body intensively to attract the intermediate host (Thomas & Ollevier, 1993). L2 larvae are capable of surviving and remaining viable in a range of environmental conditions: the survival rate of these larval stages is negatively correlated with temperature and salinity (De Charleroy & Thomas, 1989; Kirk, 2000). After ingestion by the intermediate host the nematode penetrates the digestive tract and enters the haemocoel (Blanc et al., 1992). From the 4th day on, the larvae start growing and between the 10th and

12th day at room temperature moulting to the L3 stage takes place (De Charleroy et al., 1990). L3 larvae already acquire infectivity four days after the infection of the intermediate host and their infectivity continues over 30 days (Nagasawa et al., 1994). When eaten by an eel, L3 larvae reach the swim bladder wall using a trypsin-like proteinase (Polzer & Taraschewski, 1993) and develop within two weeks into L4 stages that begin to feed on the eel blood (Bonn et al., 1990). The habitation time of the larval stages in the swim bladder wall is temperature and density-dependent (Kirk, 2000). After the last moult, the preadults, which are characterized by a not yet functioning gonadal system, enter the swim bladder cavity where sexual dimorphism develops. Copulation between sexually mature adults takes place in the swim bladder lumen of the final host. The adults decay *post mortem* in the swim bladder. The L1 larvae develop into the L2 stage in the uteri of the mated females (De Charleroy et al., 1990). At oviposition, motile L2 larvae are still surrounded by the L1 cuticle, which is shed in the haemocoel of the intermediate host (Blanc et al., 1992).

In European waters the development of *A. crassus* takes approximately three to five months (De Charleroy et al., 1990; Moravec et al., 1994a), under optimal conditions (20 - 21°C), however, less than two months. (De Charleroy et al., 1990; Kennedy & Fitch, 1990). In comparison, in the native range the life cycle requires approximately one year (Egusa, 1979). The factors limiting the spread of the parasite are:

- low water temperature in the more northern boreal regions as reported for Scandinavia and Iceland. In regions such as Iceland the parasite has not been observed at all (Höglund & Andersson, 1993; Thomas & Ollevier, 1993; Haenen et al., 1994b; Knopf et al., 1998). Temperatures below 4° C retard development from the third to fourth stage larvae, (Kirk, 2003), although viability is maintained (Knopf et al., 1998).
- high salinity in the marine environment (Dekker & Van Willigen, 1989; Nielsen, 1997; Kirk, 2000). Free living L2 larvae are unable to survive for prolonged periods in water of high salinity (De Charleroy et al., 1987). Additionally, the availability of intermediate hosts in sea water is restricted. Besides *Eurythemora affinis* there is no intermediate host of *A. crassus* known to be common in salt water environments (Kirk et al., 2000). However, salinity does not restrict development entirely. It can be completed in salt water once the parasite has been ingested by the intermediate, paratenic or final host (Höglund & Andersson, 1993; Reimer et al., 1994; Kennedy et al., 1996; Sures et al., 1999a). Adult parasites can survive and produce eggs in eels in sea water up to six months (Kirk et al., 2000).

1.2.3.1 The final hosts

The European and Japanese eels belong to the paraphyletic superclass Osteichthyes (bony fish) and to the class Actinopterygii, which constitutes a monophyletic group of ray-finned fishes (Figure 5).

Phylum: Chordata
Superclass: Osteichthyes
Class: Actinopterygii
Subclass: Neopterygii
Infraclass: Teleostei
Superorder: Elopomorpha
Order: Anguilliformes
Family: Anguillidae
Genus: *Anguilla*
Species: *A. anguilla*
A. japonica

Figure 5: Systematic classification of *Anguilla anguilla* and *Anguilla japonica*.

The subclass Neopterygii represents a very successful group, members of which can swim rapidly and are very effective predators. Nowadays, they are the most numerous group of fish on earth. The superorder Elopomorpha is characterized by the unique leptocephalus larva (Figure 6). The order Anguilliformes consists of four suborders, 19 families, 110 genera and approximately 800 species. The monotypic family Anguillidae contains the freshwater eels, which are found in most tropical, subtropical and temperate areas except for the South Atlantic and the west coasts of North and South America (Watanabe 2003). There are 18 - 19 species/subspecies in this family, all of the genus *Anguilla* (Watanabe, 2003; Aoyama, 2009; Teng et al., 2009), six of which are known to be suitable final hosts for *A. crassus*.

The distribution of *A. anguilla* includes the Atlantic coast from Scandinavia to Morocco and rivers of the North Atlantic, Baltic and Mediterranean countries (Tesch, 2003). It was also introduced to Asian countries for aquaculture including China, Japan, Malaysia and Taiwan (Nielsen & Esteve-Gassent, 2006). *A. japonica* lives in the coastal waters of East Asia and was reported in Japan, Taiwan, Vietnam, Korea, the East China Sea and the northern Philippines (state on 10.2011, http://www.fishbase.org/summary/Anguilla-japonica.html). It also represents an important aquaculture species.

All Anguillidae are catadromous and semelparous, meaning they spend their lives in freshwater rivers, lakes, or estuaries and return to the ocean to spawn where they die after reproduction (Tesch, 2003) (Figure 6).

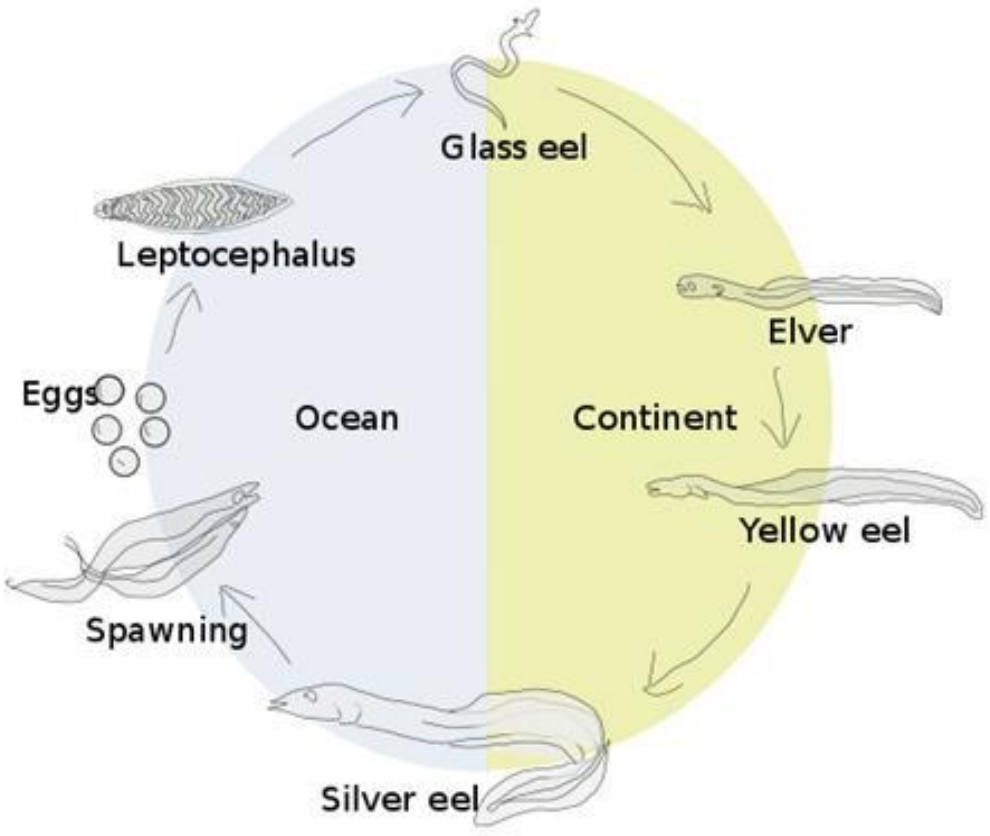

Figure 6: Developmental cycle of *Anguilla* ssp. (after Dekker (2008)).

It is commonly accepted that reproduction of European eels takes place in the Sargasso Sea area, where matured silver eels lay their eggs. Leptocephali larvae migrate towards the eastern European continental shelf edge using the Gulf Stream and North-Atlantic Drift (Tesch, 2003; Edeline et al., 2005).The spawning area of the Japanese eel is located in the waters west of the Mariana Islands in the region around 15°N, 140°E in the North Equatorial Zone. The leptocephali drift from their spawning grounds with the North Equatorial Current and then the Kuroshio Current, finally reaching the coasts of Northeast Asia (Cheng & Tzeng, 1996; Miller et al., 2002). After undergoing a first metamorphosis into glass eels, they migrate across the continental shelf to estuaries and continental waters. The eels of progressively larger size become pigmented changing colour to the yellow-eel stage. Yellow eels may live in freshwater or inshore marine and estuarine areas (Tesch, 2003) where they feed on insects, worms, molluscs, crustaceans and fish. This stage lasts usually for 5 to 8

years for males and 8 up to 20 years for females (Tesch, 2003). Interestingly, the oldest reported eels were over 80 years old (state on 10.2011, http://www.fishbase.org/summary/speciessummary.php?id=35). After maturation, at the end of the continental growing period, the transformation to silver eels takes place (second metamorphosis) and the eels begin to return to coastal waters.

During the last two decades of the previous century populations of the European eel declined by 90 – 99% and these of the Japanese eel by 80 %. The recruitment of the American eel *A. rostrata* has ceased as well (Castonguay et al., 1994). Other eel species, including Australian and New Zealand eels (*A. dieffenbachii* and *A. australis*), also showed a beginning decline (Dekker, 2008).

A. anguilla possesses the status of a critically endangered species (CR) on the red list of threatened species (state on 04. 2010, http://www.iucnredlist.org/apps/redlist/details/60344/0). There are several factors playing a role in the decline of European eel resources. On the one hand, there are abiotic factors such as extensive overfishing, migration inhibitors like dams, water power plants, embankments and restocking (Dekker, 2008). On the other hand, biotic factors such as diminished fat stores due to insufficient food supplies in the inland waters, predators such as cormorants (Brämick et al., 2008), anthropogenic contamination with xenobiotics (heavy metals, pollution, herbicids, pesticids, PCB) or global change (Dekker, 2008) dramatically influence the survival of the eels. Other important factors are viral infectious diseases like *Herpesvirus anguillae* (HVA) or Eel Virus European X (EVEX), which may negatively influence the endurance of swimming eels and hence their ability to migrate (Chang et al., 2002; Van Ginneken et al., 2005; Jakob et al., 2009a) and parasites - among others *A. crassus* (Fazio et al., 2008a).

A comprehensive review compiling actually known parasites of *A. anguilla* was published by Jakob et al. (2009b). The check-list consists of a total of 161 parasite taxa from 30 European and North African countries. The protozoan parasite species consisted of the following classes (in parentheses number of species): Mastigophora (3), Apicomplexa (3), Myxozoa (13) and Ciliophora (8), and one species of an uncertain taxonomic position (*Dermocystidium anguillae*). The metazoan parasite species consisted of the following platyhelminth classes: Digenea (39), Monogenea (8), Cestoda (20), Nematoda (38), Acanthocephala (15) and the phyla: Arthropoda (9), Annelida (2) and Mollusca (1 genus). Trematodes and nematodes are the most abundant taxonomic groups. Twenty parasites, including *A. crassus*, were

described as eel specialists (species that parasitize only or almost only on or in eels) (Buchmann et al., 1987b; Wierzbicka, 1994; Thielen, 2005; Kennedy et al., 2006; Kristmundsson & Helgason, 2007). Among these specialists, another invasive parasites, *Pseudodactylogyrus bini* and *P. anguillae,* (Monopisthocotylea: Pseudodactylogyridae), play a very important role for the European eel. Both species are indigenous to East Asia and Australia (Sures et al., 1999b) and were probably introduced to Europe with imports of Japanese eels (Koie, 1991). The monogeneans have attracted the attention of European fish parasitologists since the early 1980s, as their pathogenic effects to European eels in aquacultures (including the mortality of heavily infected individuals) have been reported continuously (Buchmann et al., 1987b). The susceptibility of the European eel to these invasive parasites, which feed on mucus, blood and epithelial cells on the gill surface (Buchmann et al., 1987a), is often explained by the lack of coevolution in this relatively young host-parasite system (Taraschewski, 2006). Since the damaging impact of the monogeneans on European eel farming was determined, several authors have dealt with various methods of abatement and prevention of the parasite in aquaculture (Buchmann et al., 1987b, 1990; Buchmann & Bjerregaard, 1990; Mellergaard, 1990; Schmahl, 1991; Waller & Buchmann, 2001). The literature available suggests that the complete elimination of the parasites is almost impossible (Buchmann et al., 1987b; Buchmann & Bresciani, 1994), making them a very serious problem especially in aquaculture systems or closed water bodies.

1.2.3.2 The intermediate hosts

Different species of low-order crustaceans belonging to the Copepoda, Ostracoda and Amphipoda serve as intermediate hosts for *A. crassus* (Table 3). It is known that the larval stages of the parasite influence the behaviour of the copepods, making them move more slowly and occupy epibenthic zones in the water body (Kirk, 2000). Intermediate hosts are eaten mainly by younger eels (De Charleroy et al., 1990). As the eels grow, the size of their prey increases (Neveu, 1981) and paratenic hosts play the main role in dispersal and survival of the nematode to larger eels (Kirk, 2003).

Table 3: Species that serve as intermediate hosts for *Anguillicola crassus* with references.

Intermediate host	Systematic group	Reference
Macrocyclops albidus	Copepoda, Cyclopoida	De Charleroy et al., 1987; Kennedy & Fitch, 1990
Macrocyclops fuscus	Copepoda, Cyclopoida	De Charleroy et al., 1987

Intermediate host	Systematic group	Reference
Paracyclops fimbriatus	Copepoda, Cyclopoida	De Charleroy et al., 1987
Eucyclops serrulatus	Copepoda, Cyclopoida	De Charleroy et al., 1987; Nagasawa et al., 1994
Eucyclops macruorides	Copepoda, Cyclopoida	De Charleroy et al., 1987
Cyclops strenuus	Copepoda, Cyclopoida	De Charleroy et al., 1987
Cyclops vicinus	Copepoda, Cyclopoida	De Charleroy et al., 1987; Kennedy & Fitch, 1990
Acanthocyclops robustus	Copepoda, Cyclopoida	De Charleroy et al., 1987
Acanthocyclops vernalis	Copepoda, Cyclopoida	De Charleroy et al., 1987
Diacyclops bicuspidatus	Copepoda, Cyclopoida	De Charleroy et al., 1987
Cypria ophthalmica	Ostracoda	Petter et al., 1990
Diaptomus gracilis	Copepoda, Cyclopoida	Kennedy & Fitch, 1990
Gammarus pulex (juvenile)	Gammaridae	Kennedy & Fitch, 1990
Eurytemora affinis (euryhaline: salt, brackish, fresh water)	Copepoda, Calanoida	Kennedy & Fitch, 1990
Thermocyclops hyalinus	Copepoda, Cyclopoida	Nagasawa et al., 1994
Thermocyclops cf. crassus	Copepoda, Cyclopoida	Knopf et al., 1998
Mesocyclops leuckarti	Copepoda, Cyclopoida	Knopf et al., 1998

1.2.3.3 Paratenesis

Under natural conditions, fully grown yellow eels become infected by *A. crassus* mainly through ingesting paratenic hosts (Haenen & Van Banning, 1990; Höglund & Thomas, 1992; Thomas & Ollevier, 1992a; Pazooki & Szekely, 1994; Szekely, 1994; Moravec, 1996). Kennedy et al. (2006) have argued that eels in nature may be more closely associated with plankton than is usually assumed. Nevertheless, the importance of paratenic hosts in the dispersal and survival of the parasite seems to be reliably determined. Available studies indicate that in general all fish species can potentially act as paratenic hosts for *A. crassus*. At least 34 fish species (De Charleroy et al., 1990; Haenen & Van Banning, 1990; Petter et al., 1990; Blanc et al., 1992; Reimer et al., 1994; Szekely, 1994; Wlasow et al., 1998; Sures et al., 1999a; Rolbiecki, 2002) and one mollusc (Moravec, 1996) have been already defined as paratenic hosts for *A. crassus*.

The paratenic hosts react to the infection with *A. crassus* at a species specific level (Szekely, 1996). After ingestion by a paratenic host, third-stage juveniles stop developing (in cyprinids), or moult into fourth-stage juveniles or pre-adults (in percids, sticklebacks and gobids). The site of habitation, further moulting and viability of these juveniles varies according to fish

species. The larval stages are often rapidly killed by the host's immune response and loose their infectivity (Kirk, 2003).

The mechanism of the cellular host reaction was generalized by Szekely (1996) and runs in following steps:

- a loose layer consisting of macrophages adheres to the larva and gradually surrounds it
- the macrophage-layer becomes thicker, the macrophages change into epithelioid cells and surround the still living larva in the form of a thick cell layer
- epithelioid cells become surrounded by a connective tissue capsule (nodule). Inside this capsule dead and alive larvae could be found
- in the advanced phase both the larvae and the epithelioid cells undergo necrosis within the connective tissue capsule and the inside of the capsule is filled by amorphous debris.

1.2.4 Morphology

The first morphological study on immature and mature adults of *A. crassus* based on light microscopy was provided by Kuwahara et al. (1974). After the introduction of the nematode to Europe Taraschewski et al. (1987) completed the survey using light and scanning electron microscopy. Later, an extensive study of the larval stages providing morphometrical details was published by Blanc et al. (1992). The summarized descriptions of developmental stages (see below) proposed by the authors were widely accepted by other parasitologists.

The L2 larval stage that is contained in the mature egg is still surrounded by the first stage cuticula (L1 sensu Geets et al. (1992)). In the intermediate host the second moult begins once the L2 larvae reach the length of 690 µm. The third moult could never been observed empirically as it takes place in the swim bladder wall of the end host. There are no obvious morphological characters differentiating the L3 and L4 stages using light microscopy. Due to the fact that the development to L4 should happen at a larval length of 1.2 - 1.6 mm, all larvae with a body length exceeding 1.5 mm are usually classified as L4 (Knopf & Mahnke, 2004). Noteworthy, Blanc et al. (1992) have distinguished between male and female L4 larvae. The last moult should take place in 1.9 to 3.9 mm long individuals. Preadults (L5 sensu Bonn et al. (1990)) and adults differ from the fourth stage larvae mainly by their well-sclerotized buccal capsule, which is provided anteriorly with one row of 17 to 22 (Blanc et al., 1992) or 22 - 28 (Taraschewski et al., 1987) large circumoral teeth. The worms suck blood

with their muscular oesophagus that consists of three lobes which are anteriorly divided into two parts. The anterior part of the oesophagus is provided with a well developed valvular apparatus. The mouth is surrounded by two dorsolateral and two ventrolateral cephalic papillae and two lateral aphids (Taraschewski et al., 1987). A sophisticated description of the structure of oesophagus and buccal capsule was provided by Bruňanská et al. (2007, 2010).

Adult worms are sexually dimorphic. They are covered by a soft wrinkled cuticle. In the male parasites the seminal vesicle is well defined, whereas in the females the uteri and the opened vulva are clearly visible. Six pairs of caudal papillae are present in the male and the cloaca is prominent (Taraschewski et al., 1987).

The following morphological features are usually measured and described in the literature: length and width of the body, oesophagus and buccal capsule, distance between the nerve ring and the anterior extremity, distance between the excretory pore and the anterior extremity, prominent cloacal process (male), distance between vulva and the posterior extremity (female), rectal glands, tail, eggs without larvae, eggs with larvae and eggs in the lumen of the swim bladder (Taraschewski et al., 1987; Rolbiecki, 2008). Comparisons between measurements of different authors are often pointless as the methods applied during analyses, including the fixation media used to preserve or clear the nematodes or the type of material examined (fresh, frozen, or chemically treated), can significantly impact the results. Further, the nematodes have different dimensions in waters of different salinity (Rolbiecki, 2008). *A. crassus* originates from fresh water, which is supposed to provide optimal conditions, leading, for example, to larger dimensions compared to individuals from brackish or saline waters. Additionally, the dimensions of the worms are depended on the size of the swim bladder of the host and infection intensity (Ashworth & Kennedy, 1999; Fazio et al., 2008b).

1.2.5 Genetic aspects

The first molecular approaches dealing with the 18S rRNA gene of *A. crassus* were undertaken to determine the phylogenetic affiliation of the Anguillicolidae within the Nematoda (Hirose et al., 1998; Wijová et al., 2006; Nadler et al., 2007) (see chapter 1.2.1). Later, Lätsch (2010) isolated nuclear and mitochondrial DNA barcoding genes (18S rDNA, 28S rDNA D2-D3 and Cox1), which were extremely useful for the identification of anguillicolid nematodes to the species level. The sequences of 28S rDNA D2-D3 and cytochrome *c*

oxidase subunit I (Cox1) proved to be useful for identification of different populations of the parasite (Lätsch, 2010).

Rahhou et al. (2005) using random amplified polymorphic DNA (RAPD) markers suggested that European populations of *A. crassus* from the Mediterranean Sea differed from those of the Atlantic and the North Sea. Moreover, the absence of a significant correlation between genetic and geographic distances supported a multiple introduction scenario for this parasite. Alternatively, using seven polymorphic short tandem repeats (STR) and one mitochondrial marker (cytochrome *c* oxidase subunit I, Cox1), a single invasion from Taiwan to Europe was postulated with a subsequent loss of genetic diversity due to random drift (Wielgoss et al., 2008). For comparison, the nematodes in North America are thought to be of Japanese origin (Wielgoss et al., 2008).

1.2.6 Impact of *Anguillicola crassus* on the final hosts

1.2.6.1 Pathology

In the native range of the parasite no pathogenic effects to the Japanese eel are known, which is traditionally explained with a long reciprocal evolutionary coadaptation within this stable host-parasite system (Egusa, 1979; Taraschewski, 2006). Similarly, no pathological impacts were reported in the Australian eel species that have coevolved with their parasites (Kennedy 1994). In contrast, a wide variety of pathological changes has been observed in the European eel. The virulence of the nematode to the European eel is considered to be related mainly to:

- the blood-sucking activity of the (pre)adult worms and migration of the larva through host's tissues resulting in mechanical damage (Egusa, 1979; Van Banning & Haenen, 1990; Molnár, 1993; Molnár et al., 1993; Haenen et al., 1994a, 1996; Kirk et al., 2000; Nimeth et al., 2000; Würtz & Taraschewski, 2000). According to Molnár et al. (1993) migrating larvae cause more substantial pathogenic changes in the swim bladder wall than blood sucking imago stages in the lumen
- decay of parasite bodies that alters the chemical composition of the swim bladder gases in infected fish (Würtz et al., 1996).
- induction of a general stress response resulting in changes in the eel's physiology (Sures & Knopf, 2004b; Sures et al., 2005; Palstra et al., 2007).
- alternations in the metabolism (Gollock et al., 2004), osmoregulation (Fazio, 2007; Fazio et al., 2008a), endocrine system (Lefebvre & Crivelli, 2004), growth and

condition of eels (Liewes & Schaminee-Main, 1987; Bonn et al., 1990; Orecka et al., 1995; Baruš et al., 1999a), and element concentration (mainly Fe) in the tissues of infected eels (Baruš et al., 1998, 1999a; b).

Massive infestation with the parasite leads to degeneration and functional disorder of the swim bladder, an organ that plays an important osmoregulatory, hydrostatic and partly respiratory role. Repeated injuries caused by the nematode initiate intensive formation of connective tissue (fibrosis) that results in thickening of the swim bladder wall (up to ten times) (Van Banning & Haenen, 1990; Molnár et al., 1993, 1995; Haenen et al., 1994a). Symptomatically, loss of appetite, emaciation, hanging near the surface of the water, open skin ulcers and lesions, and a red and swollen anus are characteristic of anguilliculosis (Egusa, 1979; Liewes & Schaminee-Main, 1987; Van Banning & Haenen, 1990; Nagasawa et al., 1994). Anal redness should be related to the abundance of the nematode and was proposed as a simple, non-invasive diagnostic tool for the estimation of infection parameters (Crean et al., 2003). Infected eels are an easier prey for larger predators (Barse & Secor, 1999) and should be relatively more vulnerable to recapture in pound nets and capture by commercial trawlers (Sjöberg et al., 2009).

Anguillicolosis is believed to play a role in the decline of the European eel stocks (Dekker, 2008). Most probably, heavily infected eels are not able to reach their spawning grounds which lie at depths of 400 - 700 m, in about 5,500 km distance from Europe (Koie, 1988; Van Banning & Haenen, 1990; Würtz et al., 1996; Nimeth et al., 2000; Kirk, 2003). Unsuccessful vertical and horizontal spawning migration is considered to be related to the serious damage of the swim bladder resulting in an impairment of its functionality. The swim bladder participates in oxygen metabolism of the eel (Molnár, 1993). Würtz et al. (1996) showed experimentally that *A. crassus* alters the mechanism of gas secretion. High adult parasite intensities reduce the proportion of oxygen in the swim bladder of eels by approximately 60% compared with uninfected eels (Würtz et al., 1996). Consequently, severely infected *A. anguilla* tend to migrate in shallower water, close to the shore (Sjöberg et al., 2009). Aberrant migration routes have been reported by Koie (1988) and handicapped vertical migration was confirmed by Palstra et al. (2007). An impaired functionality of the swim bladder results in a higher energy demand in infected eels during migration and in faster burning of the accumulated fat deposits, which leaves less fat for egg production and may result in lower egg quality (Palstra et al., 2007).

1.2.6.2 Immunology

Experimental studies with *A. anguilla* and *A. japonica* showed that *A. japonica* is significantly less susceptible to *A. crassus* in terms of the infectivity and growth rate of the parasite. The protective reaction of the Japanese eel is expressed by encapsulation and subsequent death of the L3 larvae in the gut and swim bladder walls, whereas the European eel reacts with fibrotic thickening of the swim bladder (cellular immune response), which is believed to prevent further settlement and survival of *A. crassus* (Van Banning & Haenen, 1990; Haenen et al., 1996; Lefebvre et al., 2002a). Generally, it is assumed that the Japanese eels develop an effective cellular and humoral immune response against the parasite or, more convincing, concomitant immunity has evolved in the course of coadaptation processes (Taraschewski, 2006). In wild European eels encapsulation of L3 larvae was also sporadically observed (Molnár et al., 1991, 1993; Haenen et al., 1994a; Molnár, 1994; Audenaert et al., 2003), but could never been proved experimentally (Haenen et al., 1989; Würtz & Taraschewski, 2000; Knopf & Mahnke, 2004; Knopf & Lucius, 2008). It is possible that the capsules observed in the naturally infected eels were from another parasite or they occur only under very high infection pressure (Heitlinger et al., 2009). Alternatively, the nodules could be only a product of the isolation of dead debris of larvae that were weak and died during their migration to the swim bladder wall (Knopf, 2006).

The role of the cellular immune response for migrating larvae is not fully explained. The tissue reaction involved in the encapsulation process in the European eel was first described by Molnár (1994): the larva is surrounded by macrophages that initiate the creation of a connective tissue capsule. If the larva dies due to starvation, movement constraints or is killed by phagocytes remains unclear. Mononuclear cells, giant cells and macrophages could also be observed around abnormally located L2 larvae (Molnár, 1994; Molnár et al., 1995). Additionally, a reaction of melanomacrophages that remove the debries and dead necrotic worm tissue of encapsulated adult worms were noticed in the subserosa of the swim bladder (Molnár et al., 1995). Haematological studies performed with wild and experimentally infected European eels have provided evidence for a cellular immune reaction based on an increased production of granulocytes (Boon et al., 1990a; Höglund et al., 1992; Van der Heijden et al., 1996; Sures et al., 2001). Knopf (2006) detected an increased migratory response of neutrophil granulocytes and monocytes and a weak reaction of eosinophil granulocytes in the presence of infectious L3 larvae, but no dead larvae were found in the swim bladder wall. Haenen et al. (1989), Würtz and Taraschewski (2000) and Knopf and Mahnke (2004) detected granulocytes and macrophages around the L3 but no inflammatory reaction and no larval mortality was observed. They assumed that these white cells phagocytize cell debris

created by the larva. So far no protective function of the leucocytes has been observed (Boon et al., 1990c; Van der Heijden et al., 1996; Knopf et al., 2008). Knopf (1999) reported an increased migratory response of phagocytes in the presence of L3 which apparently occurred irrespective of migrating larva, while other authors did not find any cellular immune reaction in the blood against larval stages in the swim bladder wall of naturally infected eels (Boon et al., 1989, 1990c; Palikova & Navratil, 2001).

There is also no proof for a role of antibody responses in immune protection, neither in the European nor in the Japanese eel (Knopf & Lucius, 2008). In the European eels infected with *A. crassus* a significant increase of B-lymphocyte content as well as in the total number of circulating lymphoid cells could be shown, but no specific antibodies could be verified (Van der Heijden et al., 1996). However, specific antibodies against antigens located in the cuticula of the adult worms were detected both in naturally infected (Buchmann et al., 1991; Höglund & Pilström, 1994, 1995; Haenen et al., 1996; Nielsen & Buchmann, 1997) and experimentally infected European eels (Knopf et al., 2000; Sures & Knopf, 2004a), but the expected mortality of adult worms remains doubtful (Békési et al., 1997). In Japanese eels antibodies are also probably directed against the cuticular antigen of adults (Ushikoshi et al., 1999). However, no protective humoral immunity against L3 of *A. crassus* in experimentally infected European eels has been detected so far. As mentioned above, the presence of larval stages induces higher cortisol levels in the European eel, which is negatively correlated with the concentration of anti - *A. crassus* antibodies (Sures et al., 2005). The authors hypothesized, that it might be a parasite survival strategy that helps or even allows the survival of *A. crassus* inside its host. It appears likely that the parasite evokes a clear stress response, which then suppresses the host's humoral immune response to adult antigens and allows its own successful infestation.

Vaccination experiments with irradiated L3 larvae conducted with both eel species have shown that exclusively the native host could restrict the burden of the parasite by an adaptive immune response (Knopf & Lucius, 2008). The European eel was not protected by acquired immunity to repeated infections with *A. crassus* (Haenen et al., 1996; Knopf, 1999). The specific humoral immune response in the European eel was characterized by a later onset comparing to its Japanese congener (Nielsen, 1999). *A. japonica* apparently possesses a strong innate and adaptive immune response, which probably retards growth of the worms (neutrophils) and reduces their infectivity. The observed adaptive immunity of *A. japonica* was definitely not related to the production of antibodies, as both eel species were able to produce them (Nielsen, 1999). Additionally, the amount of antibodies is believed to be

correlated with the number of adults and not with the larval stages of the parasite (Nielsen & Buchmann, 1997; Knopf et al., 2000; Knopf & Lucius, 2008). Further investigations, under special consideration of concomitant immunity, are needed to clarify the mechanism of immune protection by Japanese eels (Brown & Grenfell, 2001).

1.3 Thesis goals

Anguillicola crassus is one of the most successful aquatic parasitic aliens in the history of globalization. Within few decades after its introduction this nematode has reached the last stage of the invasion process *sensu* Colautti and MacIsaac (2004), i.e. it is wide spread and dominant. Suitable intermediate, paratenic and final hosts were already available in the new environment, sufficiently matching the ecological requirements of the nematode and doubtlessly supporting its invasion success. However, *A. crassus* lacks some of the typical characters of an invasive species, such as a direct developmental cycle or hermaphroditism. Furthermore, it arrived in Europe in only a single release event (Wielgoss et al., 2008), partly negating the importance of the propagule pressure model in the invasion theory, which states that introduced species become invasive only after repeated introductions (Lockwood et al., 2005). In addition, similar to other invasive species, the European population of *A. crassus* has experienced a genetic bottleneck leading to lowered genetic diversity (Wielgoss et al., 2008). This should reduce the ability of an introduced population to become established in its new environments (Allendorf & Lundquist, 2003). Such rapid invasions by taxa that do not possess typical invader traits are characteristic of species that were already pre-adapted to the new environment as they originate from a similar one (Facon et al., 2006). However, pre-adapted traits alone are unlikely to explain an invasion success, phenotypic plasticity and local adaptations are more likely to support the establishment in the new area (Suarez & Tsutsui, 2008).

Parasite invasion has attracted much less attention than the invasion of free living animals, although about 50% of all animals show a parasitic mode of living (Bush, 2001). If a dispersal jump of a parasite coincides with a host switch, the novel host may act as a substantially different environment, leading to the onset of a new host-parasite coevolution with reciprocal adaptations (Taraschewski, 2006).

Here I look at the invasive, haematophagous nematode *A. crassus* (naturally parasitizing the Japanese eel *Anguilla japonica*), which was introduced in the early 1980s from Taiwan to Europe (Neumann, 1985), colonizing populations of the European eel *A. anguilla* (Kirk, 2003; Wielgoss et al., 2008). Existing field and laboratory studies with this nematode revealed that

the worms in Europe differ from their Taiwanese conspecifics infecting the Japanese eel. Recovery, weight gain and reproductive output were significantly higher in the European eel, in contrast to larval mortality, which was lower in this host (Knopf & Mahnke, 2004). The results obtained by Knopf & Mahnke (2004) corresponded with those from the field that additionally revealed morphometric differences between the European and Taiwanese parasite populations (Münderle, 2005). These data and observations suggest that *A. crassus* and *A. japonica* have undergone a long, reciprocal coevolution resulting in low pathological impact on the adapted host. In contrast, in the novel host the lack of concomitant immunity should have brought about very different conditions for host-parasite coevolution resulting in altered infection parameters in this eel species.

I therefore hypothesized that *A. crassus* is undergoing genetic divergence in ecological time due to 30 years of spatial isolation, equivalent to about 30 - 60 generations of the parasite in the European eel. To support this hypothesis I carried out a common garden experiment under a reciprocal transplant design (Nuismer & Gandon, 2008) to determine, whether differences in recovery, developmental dynamics, reproductive potential and morphology between the European and Asian nematode populations occur while harboured in the native and colonised hosts, and if these differences are genetically based or are plastic responses to the new living conditions.

2 Materials and methods

2.1 Experimental design

I conducted a common garden experiment under a reciprocal transplant design infecting *Anguilla japonica* and *A. anguilla* with *Anguillicola crassus* originating from Poland, Germany and Taiwan (Figure 7). Common garden experiments are a widespread method allowing to distinguish between genetic and plastic conditioned traits by comparing different populations under the same environmental conditions (Nuismer & Gandon, 2008). Eels of each species were infected with 50 L3 larvae from each parasite population. At 25, 50, 100 and 150 days post infection (dpi) the eels were dissected and parasitologically examined. The dissection periods were chosen after De Charleroy et al. (1990). The experiments were performed between the 15.01.07 and 09.03.2009.

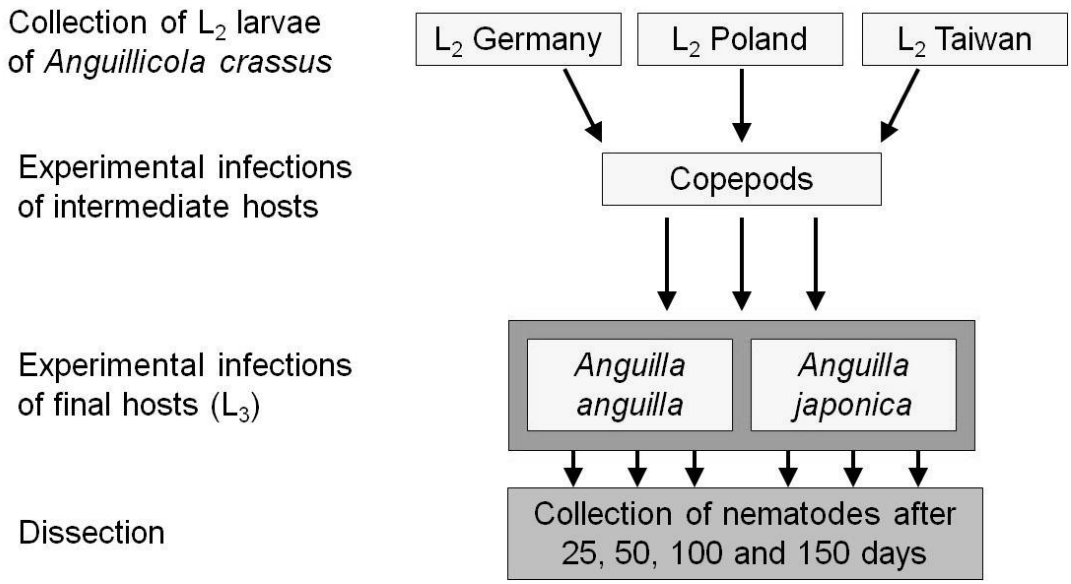

Figure 7: Experimental design.

2.2 Collection of L2 larvae and infection of the intermediate host

The L2 larvae (Figure 8) used in the experiment were collected in autumn 2006 and 2007 from the swim bladders of wild yellow and silver eels from the Rhine River near Karlsruhe in Germany, the Kao – ping River and adjacent aquacultures in Taiwan and the Lake Śniardwy

in Poland. They were stored at 4°C for no longer than two weeks before the cyclopoid copepods of the species *Cyclops vicinus* (Figure 9) were infected.

Figure 9: Eggs (right arrow) and hatched L2 larvae (left arrow) of *Anguillicola crassus*. Center - left the apical end of an adult worm.

Figure 8: *Cyclops vicinus* infected with L3 larvae of *Anguillicola crassus*. The arrows point the L3 larvae.

The copepods were collected from a pond in the Botanical Garden of the Karlsruhe Institute of Technology (KIT), which is free from eels and consequently from the parasite. The copepods were infected in microtiter plates (Carl Roth, Rotilabo®, 9291.1, U-profile, 345 µl/well) (Figure 10).

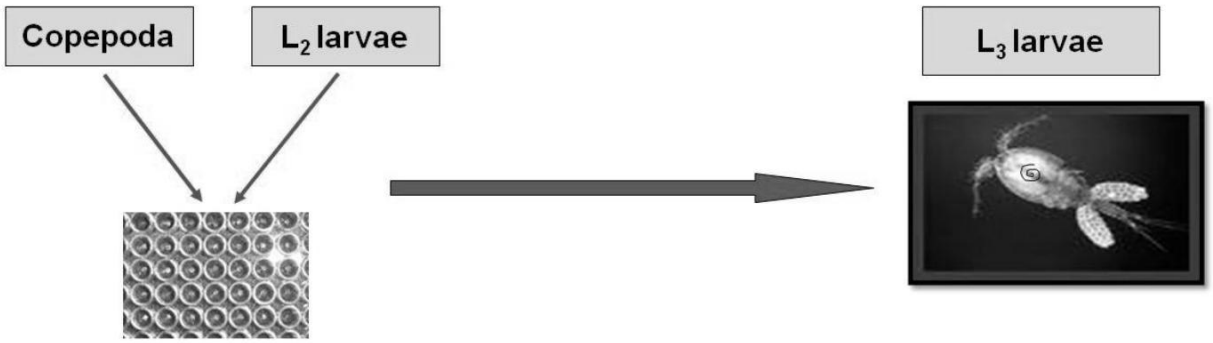

Figure 10: Infection of the intermediate host.

The infection intensity was ~ 10 L2/copepod. The intermediate hosts were fed with yeast twice a week. After one week they were removed from the microtiter plates and placed into oxygenated 20-liter tanks filled with tap water with a 12:12 photoperiod at 21°C. At 21 dpi the L3 larbae were harvested with a tissue potter in RPMI-1640 medium using a modified procedure proposed by Haenen et al. (1994b). L3 larvae were counted under a binocular

microscope (Semi 2000, Zeiss, Germany) in a round bottomed microtiter plate and suspended in approximately 100 µl RPMI-1640 cell culture medium. They were stored temporary at 4°C before the eels were infected.

2.3 Experimental conditions

Uninfected yellow eel stages of *A. anguilla* (Figure 11) were obtained from the Albe-Fish-farm in Haren-Rütenbrock, Germany, and transported in aerated tanks to the Zoological Institute of the KIT. *A. japonica* (Figure 12) were caught at the glass-eel stage in the Kao-ping River estuary, Taiwan, by a professional fisherman and transported in aerated bags to the KIT by airmail.

Figure 11: *Anguilla japonica* in the yellow stage.

Figure 12: *Anguilla anguilla* in the yellow stage.

The absence of *A. crassus* was confirmed by dissection of ten randomly chosen eels of each species. Before the experiments started, *A. japonica* were fed with commercial fish pellets (Dan-Ex 2848, Dana Feed A/S Ltd, Horsens, Denmark) until the eels reached the yellow eel stage. All eels were kept in experimental tanks for several weeks before the experiments started. In order to exclude co-infection with *Pseudodactylogyrus* spp. the eels were treated twice at a six day interval with praziquantel (Tremazol, Sera).

Infected eels were kept in 160-liter tanks in groups of 20 individuals at a constant temperature of 22°C and a 12:12 photoperiod. At 25, 50, 100 and 150 dpi five eels from each tank were randomly chosen and dissected resulting in 20 eels per dpi-group at the end of the experiment. The tanks were continuously provided with fresh, oxygenated water by a recirculation system. The eels were fed every day *ad libitum* with commercial fish pellets

(Dan-Ex 2848, Dana Feed A/S Ltd, Horsens, Denmark). As the animals are night-active and have a benthic life style, polypropylene tubes were provided as a hiding-facility.

2.4 Infection and investigation of eels

The eels were infected via a stomach tube (1.5 mm diameter) (Figure 13) after the procedure described by Bonn et al. (1990). After decapitation and despinalization, the eels were measured to the nearest 1.0 mm in order to estimate the size of the niche available for development of the parasites (Thomas & Ollevier, 1992b). The body size of the host is thought to be positively correlated with the dimensions of the parasites (Morand et al., 1996) and is considered to be important for infrapopulation regulation processes (Fazio et al., 2008b). After that the eels were weighted to the nearest 1.0 g. After dissection (Figure 13), the sex of the eels was determined by visual inspection of the gonads after Tesch (2003). Sex is an important factor influencing the immunocompetence of the hosts (Tschirren et al., 2003). The stomach, gut and swim bladder were removed and cut along their entire length.

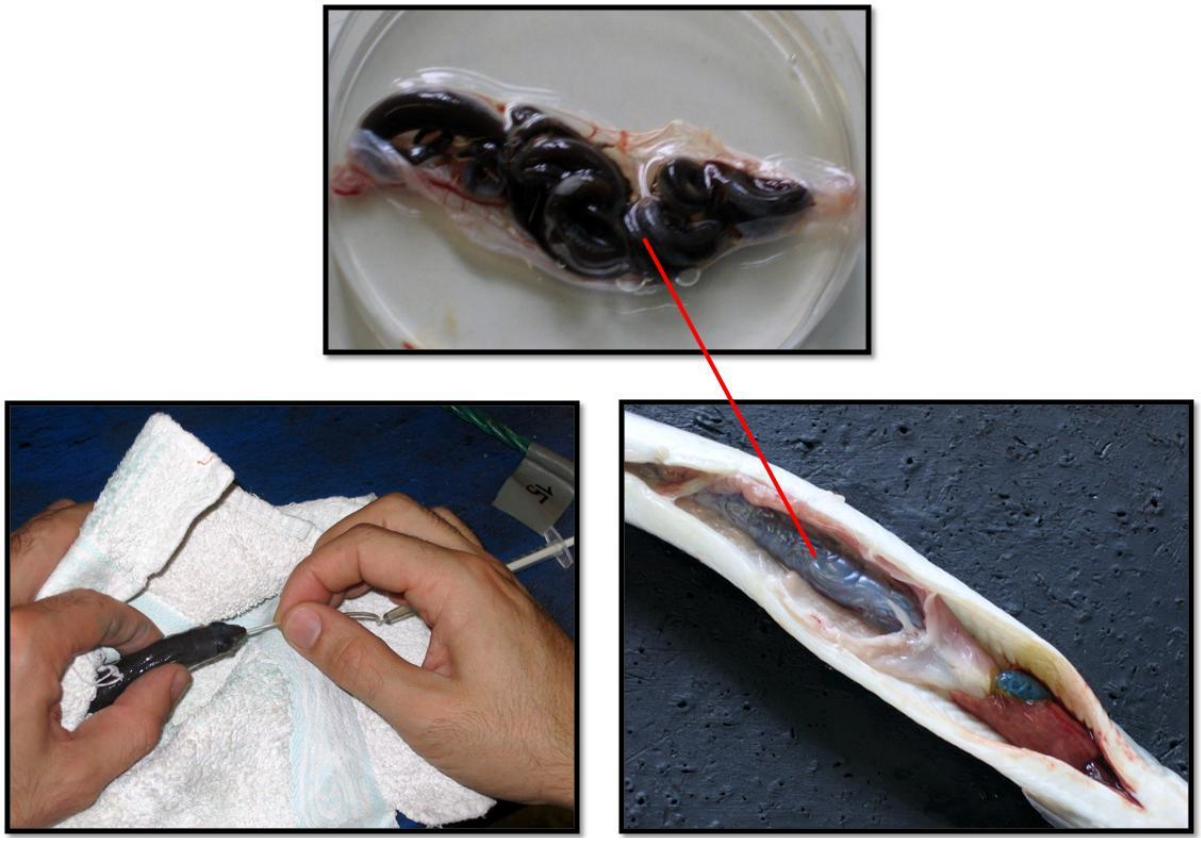

Figure 13: Infection and dissection of eels. Left: infection of an eel with a stomach tube. Right: an opened eel. At the top: an opened swim bladder of an European eel.

2. Material and methods

Adult parasites (Figures 14 - 15) were removed from the swim bladder, determined to sex and preserved in 95% ethanol for morphometric investigations. The eggs laid in each swim bladder were collected and suspended in a cap filled with 40 ml tap water.

Figure 14: An adult female *Anguillicola crassus* collected from an European eel.

Figure 15: An adult male *Anguillicola crassus* collected from an European eel. In the background eggs and L2 larval stages can be seen.

The swim bladder, stomach and gut walls were searched for the larval stages by squashing them between two perspex plates and using transmitted light under a binocular microscope (Semi 2000, Zeiss, Germany) (Figures 16 - 17).

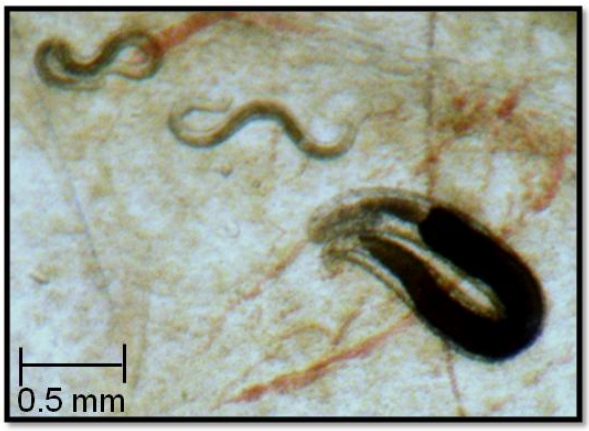

Figure16: L3 larvae (on the left and in the middle) and a L4 larval stage (on the right) of *Anguillicola crassus* in the swim bladder wall of an European eel.

Figure 17: An encapsulated larval stage of *Anguillicola crassus* in the swim bladder wall of an Japanese eel.

Identification of all larval stages, encapsulations in the swim bladder wall, determination of the sex of adult worms and measurements of adults were undertaken using a binocular microscope (Semi 2000, Zeiss, Germany). The European eels that did not show any signs of infection at 25 and 50 dpi (under assumption of infection failure) were not considered in the statistical analysis.

2.5 Recovery, developmental dynamics and reproductive potential

The swim bladders were investigated for eggs, L3, L4 and adults as well as dead larvae and dead adults. In order to quantify the eggs, 5 samples of 2 ml were taken from each 40 ml cap. The means were extrapolated to the whole volume of each sample and the mean number of eggs per eel was estimated. Larvae smaller than 1.5 mm were counted as L3, bigger larvae as L4 (Knopf & Mahnke, 2004). All specimens with developed sexual organs were classified as adults. Based on the number of each life history stage recovered at each dpi, the recovery, dynamics of development and reproductive potential were estimated for each population in each eel species. Recovery is the ability of the parasite to infect the host expressed as the mean percentage of the recovered worms from the total worms applied. Developmental dynamics refers to the speed of moulting to the next developmental stage (L3 → L4 (dead larvae) → adults → dead adults) and is expressed as the mean number of particular life history stage found at each dpi. Reproductive potential is the mean number of eggs per eel at each dpi.

2.5.1 Statistics

2.5.1.1 Descriptive statistics and non-parametric Mann-Whitney U-tests

The experiment resulted in 24 independent data sets (3 populations*4 dpi*2 eel species) for the recovery and 144 independent data sets (3 populations*4 dpi*2 eel species*6 developmental stages) for the developmental dynamics and reproductive potential. These were primarily described by the mean ($\bar{x}$), standard deviation (SD), standard error (SE) and minimum and maximum values. An exploratory data analysis was performed to check whether data was normally distributed or not. To test for differences between groups a non-parametric Mann-Whitney U-test for unpaired two-sample data sets was chosen. Due to

overdispersion, parasite populations were compared pair wise separately for each dpi and each eel species. These statistics were executed using SPSS 17 (PASW Statistics 17). The significance was assumed if $p<0.05$.

2.5.1.2 Fixed-effects linear models

The models were performed separately for each term acting as response variable (recovery rate, eggs or L2, L3, L4, adults, dead adults and dead larvae). As no repeated data were recorded from a single eel specimen, all explanatory variables were assumed to have fixed effects on each response variable. All data were counts and were modelled as numerical variables. Each maximal model started with a set of basic explanatory variables and a set of additional, for each model characteristic, explanatory variables. The basic explanatory variables consisted of: eel species, parasite population, dpi (as a continuous variable), eel length, tank number and interactions between eel species, parasite population and dpi. In the models involving the dynamics of development additional interactions between each developmental stage, eel species and population were inserted. Primarily, two series of tests either with German or with Polish populations were conducted in the European eel as a reference group (Taiwan-Germany-Poland models). Additionally, both European populations were pooled to a single group (Taiwan-Europe models) and treated with an analogous statistical procedure as differences were not found between these populations. Each model was fitted by stepwise simplification (marginal ANOVA) starting from maximal models. In order to eliminate correlations between the intercept and the slope, the equations adjusting the mean of the function modelled were estimated against the intercepts set on 25 dpi. For eggs as response variable two models were run. The big model contained all possible explanatory variables. As the highly significant explanatory variables in the big model did not convey deeper insight into interpretation of the output, a small model containing only basic explanatory variables was run. In these models the intercept was set on 50 dpi as no eggs were laid before this experimental interval and the eggs-counts were log-transformed due to their heteroscedasticity. The additional explanatory variables and explanatory variables included in each saturated maximal model after application of the simplification procedure are presented in tables 4 and 5. All statistics were conducted using R (R Development Core Team, 2009). Significance was assumed if $p<0.05$.

Table 4: Set up of the Taiwan-German-Poland fixed-effects linear models: estimated terms, response variables, additional explanatory variables and saturated models.

Estimated term	Model no	Response variable	Additional explanatory variables in the maximal models	Explanatory variables and interactions in the minimal adequate models
Recovery	1, 9	Numbers of L3 + L4 + adults recovered alive	Eel sex	Eel species Parasite population Dpi Eel species*Dpi Parasite population *Dpi Eel species*Parasite population
Development	2, 10	Number of L3 recovered alive	Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Number of dead adults Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of L4 recovered alive Number of adults recovered alive Mean length of adults Eel species*Parasite population
	3, 11	Number of L4 recovered alive	Number of L3 recovered alive Number of adults recovered alive Number of dead larvae Number of dead adults Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of L3 recovered alive Eel species*Parasite population Eel species*Dpi Parasite Population*Dpi Eel species*Parasite population*Dpi
	4, 12	Number of adults recovered alive	Number of L3 recovered alive Number of L4 recovered alive Number of dead larvae Number of dead adults Number of eggs	Eel species Parasite population Dpi Eggs Dead adults Eel*Dpi Parasite population*Dpi Parasite population*Dead adults Eel species*Eggs Parasite population*Eggs
	5, 13	Number of dead adults	Number of L3 recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of dead larvae Number of eggs Number of adults recovered alive Eel species*Dpi Eel species*Dead larvae Parasite population*Eggs Parasite population*Adults
	6, 14	Number of dead	Number of L3	Eel species

Estimated term	Model no	Response variable	Additional explanatory variables in the maximal models	Explanatory variables and interactions in the minimal adequate models
		larvae	recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead adults Mean length of adults recovered alive Number of eggs	Parasite population Dpi Number of dead adults Eel species*Parasite population Eel species*Dpi Eel species*Dead adults
Reproductive potential	7 , 15 (big model)	Log number of eggs	Number of L3 recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Number of dead adults Mean length of adults recovered alive	Eel species Dpi Adults Number of dead adults Mean length of adults recovered alive Eel species*Dpi
	8 , 16 (small model)	Log number of eggs	Number of L3 recovered alive Number of L4 recovered alive Number of dead larvae	Eel species Parasite population Dpi Eel species*Dpi

Table 5: Set up of the Taiwan-Europe fixed-effects linear models: estimated terms, response variables, additional explanatory variables and saturated models.

Estimated term	Model no	Response variable	Additional explanatory variables in the maximal models	Explanatory variables and interactions in the minimal adequate models
Recovery	17	Numbers of L3 + L4 + adults recovered alive	Eel sex	Eel species Parasite population Dpi Eel species*Dpi Parasite population *Dpi Eel species*Parasite population
Development	18	Number of L3 recovered alive	Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Number of dead adults Mean length of adults recovered alive	Eel species Parasite population Dpi Number of L4 recovered alive Mean length of adults Adults Eel species*Parasite population

Estimated term	Model no	Response variable	Additional explanatory variables in the maximal models	Explanatory variables and interactions in the minimal adequate models
			Number of eggs	
	19	Number of L4 recovered alive	Number of L3 recovered alive Number of adults recovered alive Number of dead larvae Dead adults Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of L3 recovered alive Eel species*Parasite population Eel species*Dpi Parasite population*Dpi Eel species*Parasite population*dpi
	20	Number of adults recovered alive	Number of L3 recovered alive Number of L4 recovered alive Number of dead larvae Number of dead adults Number of eggs	Eel species Parasite population Dpi Number of dead adults Number of eggs Eel*Dpi Parasite population*Dpi Parasite population*Dead adults Eel species*Eggs
	21	Number of dead adults	Number of L3 recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of dead larvae Number of eggs Number of adults recovered alive Eel species*Dpi Eel species*Dead larvae Parasite population*Eggs Parasite population*Adults
	22	Number of dead larvae	Number of L3 recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead adults Mean length of adults recovered alive Number of eggs	Eel species Parasite population Dpi Number of dead adults Eel species*Parasite population Eel species*Dpi Eel species*Dead adults
Reproductive potential	23 (big model)	Log number of eggs	Number of L3 recovered alive Number of L4 recovered alive Number of adults recovered alive Number of dead larvae Number of dead adults Mean length of adults recovered alive	Eel species Dpi Number of adults recovered alive Number of dead adults Mean length of adults recovered alive Eel species*Dpi

Estimated term	Model no	Response variable	Additional explanatory variables in the maximal models	Explanatory variables and interactions in the minimal adequate models
	24 (small model)	Log number of eggs	Number of L3 recovered alive Number of L4 recovered alive Number of dead larvae	Eel species Parasite population Dpi Eel species*Dpi

2.6 Investigations for morphology

2.6.1 Measurements

The length and the width of the body (mm), oesophagus (µm) and buccal capsule (µm) were measured at the longest and widest point (Figure 18). The adult worms were measured at 0.65x, 2.0x or 5.0x magnification by the means of a binocular microscope (STEMI 2000, Carl Zeiss) provided with a measuring eyepiece. After the body dimensions were recorded, the head was removed behind the oesophagus with a fine scalpel and covered with a Berlese-mixture (Waldeck GmbH & Co. KG, Division Chroma, 3D 101) on a glass slide. The oesophagus and buccal capsule were measured at 10x and 100x magnification by the means of a compound microscope (Axiolab, Carl Zeiss) that was also provided with a measuring eyepiece. The optical instruments were calibrated after Münderle (2005). In order to describe differences in the shape of the morphological features measured, the ratio of each measurement pair (length/width) was estimated (the smaller ratio the rounder/fatter the morphological trait). The criterion for qualification of the worms for this analysis was the good condition of morphological traits after fixation.

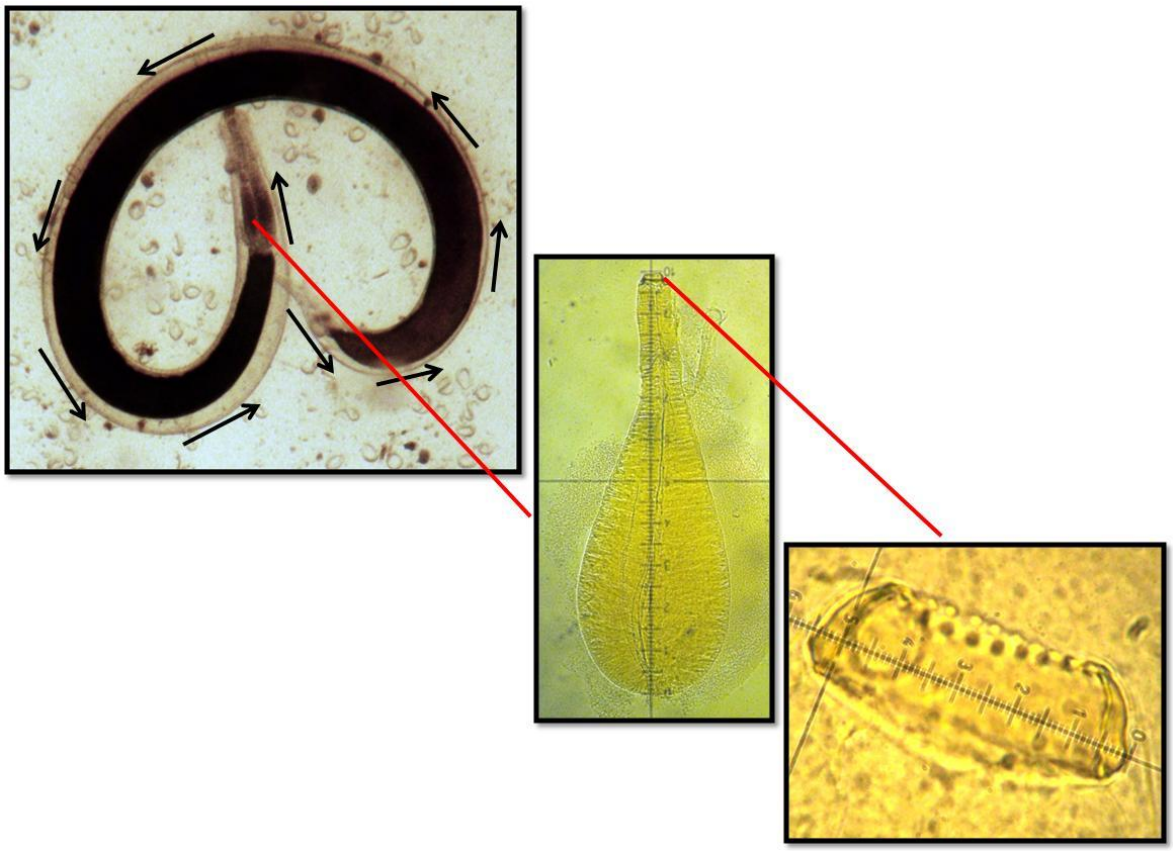

Figure 16: The measurements of the body (on the left), oesophagus (in the middle) and buccal capsule (on the right) of *Anguillicola crassus*.

2.6.2 Statistics

2.6.2.1 Descriptive statistics

The morphological investigations resulted in 432 independent data sets (3 worm populations*4 dpi*2 eel species*9 measurements*2 genders). The separation of genders was essential due to the morphometrical dimorphism of the adult worms (Münderle, 2005). Independently for each group, the mean, standard error (SE), standard deviation (SD) and minimum and maximum values were estimated.

2.6.2.2 Mixed-effects linear models

The models were performed separately for each term acting as response variable (the length, width and ratio of body, oesophagus and buccal capsule). As repeated measurements within one eel were reclaimed Mixed-effects linear models were chosen. Before the analysis started the data set was cleaned from extreme outliers based on Box and

Whisker Plots. The models were fitted by stepwise simplification (marginal ANOVA) starting from maximal models. As the number of observations at 25 dpi was statistically insufficient the equations adjusting the mean of the function modelled were estimated against the intercepts set on 50 dpi. All data were modelled as numerical variables. Eel specimen was modelled as a random effects explanatory variable, all other factors were modelled as fixed effects explanatory variables. Each maximal model started with a set of following explanatory variables: eel species, length of eel, parasite population, dpi (as a continuous variable), eel specimen, worm sex, number of L3, L4 and adult worms. Additionally, interactions between eel species, parasite population and dpi and sex of the worms and dpi and sex of the worms and eel species were allowed. The explanatory variables included in each saturated model after application of the simplification procedure are presented in table 6. All statistics were executed using R (R Development Core Team, 2009). Significance was assumed if $p<0.05$.

Table 6: Set up of the mixed-effects linear models: response and explanatory variables in the minimal adequate models after simplification.

Response variable	**Model no**	**Explanatory variables in the minimal adequate models**
Body length	1, 10	Eel species Dpi Number of adults recovered alive Number of L3 recovered alive Worm sex Eel species*dpi Dpi*worm sex Eel species*worm sex
Body width	2, 11	Eel species Dpi Number of adults recovered alive Number of L3 recovered alive Worm sex Eel species*dpi Dpi*worm sex Eel species*worm sex
Ratio body length/width	3, 12	Eel species Population Dpi Worm sex Eel species*population Eel species*dpi Dpi*worm sex Eel species*worm sex
Oesophagus length	4, 13	Eel species Population Dpi Number of adults recovered alive Number of L3 recovered alive Worm sex Eel species*dpi Dpi*worm sex

Response variable	Model no	Explanatory variables in the minimal adequate models
Oesophagus width	5, 14	Eel species Population Dpi Number of adults recovered alive Worm sex Eel species*dpi Dpi*worm sex
Ratio oesophagus length/width	6, 15	Eel species Population Dpi Worm sex Eel species*dpi
Buccal capsule length	7, 16	Eel species Population Dpi Number of adults recovered alive Number of L3 recovered alive Worm sex Eel species*dpi
Buccal capsule width	8, 17	Eel species Population Dpi Number of L3 recovered alive Worm sex Eel species*dpi Population*dpi
Ratio buccal capsule length/width	9, 18	Eel species Population Dpi Worm sex Eel species*worm sex

3 Results

This section consists of 3 parts: the first is dealing with the data from the infected eels and presents details of the length and weight of the European (*Anguilla anguilla*) and Japanese eels (*Anguilla japonica*) that were chosen for infections with *Anguillicola crassus* populations from Germany, Poland and Taiwan. The second section (recovery, developmental dynamics and reproductive potential of *A. crassus* populations in the European and Japanese eels) deals with differences between the nematode populations in terms of recovery, developmental dynamics and reproductive potential in the same eel species, presenting descriptive statistics as well as results of non-parametric tests and fixed-effects linear models. This chapter additionally considers the general infection patterns of the nematodes depending on the eel species being infected (host-dependent developmental patterns), as well as other developmental features that were neither linked to the host species, nor to the origin of the parasites (other effects: regulation of infrapopulation density). The last chapter (morphology of *A. crassus* populations in the European and Japanese eel) presents descriptive statistics referring to the dimensions of the body, oesophagus and buccal capsule of the parasite populations and the statistical analysis of the morphological measurements based on mixed-effects linear models. Analogous to the previous chapter, it is subdivided into sections specified by differences between morphological traits of the populations in the same eel species, host-dependent morphological patterns, as well as to other effects (density-dependent and host-dependent regulation of worm size; general developmental patterns) that occurred independently of the host species or parasite population.

3.1 Data from infected eels

A total of 331 European and 224 Japanese eels were randomly chosen for the trial. Eels that died prior to infection with *Pseudodactylogyrus* sp. *Trichodina* sp., *Ichthyophthirius* sp. *Saprolegnia* sp., which were probably introduced to the laboratory with fish equipment used in the outdoor water bodies, were not considered in the statistical analysis. Subsequently, 239 European eels and 216 Japanese eels successfully participated in the experiment. The tables 7 and 8 present the mean, standard error (SE), standard deviation (SD) and minimum and maximum values of the length and weight of the European and Japanese eels used in the trial. The length and weight of the smallest European eel were 29 cm and 41 g and of the biggest European eel 45 cm and 168 g, respectively. In the case of the Japanese eels, the smallest individual was 42 cm in length and 60 g in weight and the biggest had 67 cm in

length and 365 g in weight. The fraction of female eels was substantially lower than that of the males and accounted 19.3% for the European eels and 5% for the Japanese eels.

Table 7: The mean length (cm) and weight (g) of the European eels infected with *Anguillicola crassus* from Germany, Poland and Taiwan with standard error (SE), standard deviation (SD) and minimum and maximum values. Dpi – days post infection; N – number of infected eels.

Inoculated parasite population	Dpi	Length/Weight	N	Mean	SE	SD	Minimum	Maximum
German	25	Length	20	37.5	0.5	2.1	34	42
		Weight	20	80.3	4.4	19.7	50	125
	50	Length	25	37.6	0.5	2.7	32	43
		Weight	25	81.7	3.2	16.2	51	122
	100	Length	20	37.8	0.6	2.7	35	45
		Weight	20	85.5	5.5	24.4	50	155
	150	Length	21	37.6	0.6	2.8	33	43
		Weight	21	87.0	5.4	24.6	48	151
Polish	25	Length	20	36.5	0.5	2.1	32	42
		Weight	20	76.0	3.1	14.0	55	128
	50	Length	25	38.0	0.6	3.1	32	44
		Weight	25	86.3	4.5	22.4	53	144
	100	Length	17	38.9	0.6	2.4	36	45
		Weight	17	86.1	5.7	23.6	65	165
	150	Length	22	37.9	0.3	1.4	36	41
		Weight	22	82.6	4.6	21.7	59	137
Taiwanese	25	Length	22	37.2	0.6	3.0	29	42
		Weight	22	84.6	3.3	15.4	58	120
	50	Length	20	37.7	0.7	2.9	33	43
		Weight	20	82.5	6.5	29.1	41	168
	100	Length	18	38.0	0.5	2.2	34	43
		Weight	18	83.2	3.4	14.5	65	125
	150	Length	19	37.5	0.6	2.6	33	44
		Weight	19	84.4	4.2	18.5	55	135

Table 8: The mean length (cm) and weight (g) of the Japanese eels infected with *Anguillicola crassus* from Germany, Poland and Taiwan with standard error (SE), standard deviation (SD) and minimum and maximum values. Dpi – days post infection; N – number of infected eels.

Inoculated parasite population	Dpi	Length/Weight	N	Mean	SE	SD	Minimum	Maximum
German	25	Length	16	48.2	0.7	2.9	42	54
		Weight	16	147.2	11.5	45.9	81	250

Inoculated parasite population	Dpi	Length/Weight	N	Mean	SE	SD	Minimum	Maximum
	50	Length	20	49.4	0.9	4.2	42	57
		Weight	20	168.6	13.8	61.7	70	280
	100	Length	16	49.3	0.8	3.0	45	57
		Weight	16	176.6	12.7	50.9	100	285
	150	Length	18	49.4	0.4	1.6	47	53
		Weight	18	171.9	5.5	23.3	140	230
Polish	25	Length	17	48.9	0.5	1.9	46	53
		Weight	17	165.7	7.0	28.8	110	205
	50	Length	16	49.9	0.7	2.8	44	53
		Weight	16	178.7	9.9	39.5	87	245
	100	Length	17	52.0	1.0	4.2	44	58
		Weight	17	198.5	16.8	69.4	90	320
	150	Length	18	49.0	1.4	5.9	44	67
		Weight	18	158.9	16.0	67.8	85	320
Taiwanese	25	Length	18	48.1	0.4	1.5	46	51
		Weight	18	144.9	6.4	27.0	115	195
	50	Length	20	48.4	0.5	2.2	45	54
		Weight	20	167.6	9.8	43.7	84	255
	100	Length	20	49.1	1.0	4.4	43	59
		Weight	20	155.7	12.0	53.7	60	290
	150	Length	20	51.6	1.3	5.8	42	67
		Weight	20	176.2	17.7	79.1	70	365

3.2 Recovery, developmental dynamics and reproductive potential of populations of *Anguillicola crassus* in the European and Japanese eel

3.2.1 Differences between populations of *Anguillicola crassus* in the same eel species

3.2.1.1 Differences in recovery

Recovery is the ability of the parasite to infect the host and is expressed as percentage of the recovered worms from the total worms applied. All recovered worms were found in the swim bladder of the eels. The stomach and the intestine were free from larval stages of the parasite. The mean, standard error (SE), standard deviation (SD), minimum and maximum values of recovery of the German, Polish and Taiwanese populations of *A. crassus* in the

European eel, as well as in the Japanese eel, are presented in tables 9 and 10. The high standard deviation values around the mean of recovery and the minimum and maximum recovery values suggest an overdispersion of the data indicating that individual eels were unequally infected with the parasites.

The percentage of European worms recovered in the European eel ranged from 14% (German population at 150 dpi) to 30% (Polish population at 50 dpi). In the European eels infected with Taiwanese larvae, 36% at 25 dpi and 44% at 100 and 150 dpi of inoculated worms were recovered.

Table 9: The recovery (%) of *Anguillicola crassus* from Germany, Poland and Taiwan in the European eel with standard error (SE), standard deviation (SD) and minimum and maximum values; Dpi - days post infection.

Dpi	Parasite population	Mean	SE	SD	Minimum	Maximum
25	German	26.6	2.1	9.2	2	42
	Polish	24.7	3.6	16.3	6	60
	Taiwanese	35.6	2.6	11.9	20	54
50	German	25.3	2.4	12.0	8	50
	Polish	30.3	3.5	17.7	2	78
	Taiwanese	42.2	3.5	15.4	14	66
100	German	22.5	2.9	12.9	2	54
	Polish	17.5	3.1	12.7	2	50
	Taiwanese	44.4	3.2	13.1	14	68
150	German	13.8	2.3	10.4	0	38
	Polish	21.6	3.3	15.3	0	54
	Taiwanese	43.8	4.4	19.8	10	86

In the Japanese eel infected with European larvae recovery varied from 5% (Polish population at 150 dpi) to 45% (German population at 25 dpi). In the Japanese eel infected with Taiwanese worms the fraction of recovered worms ranged between 25% at 25 dpi and 4% at 150 dpi.

Table 10: The recovery (%) of *Anguillicola crassus* from Germany, Poland and Taiwan in the Japanese eel with standard error (SE), standard deviation (SD), and minimum and maximum values; Dpi - days post infection.

Dpi	Parasite population	Mean	SE	SD	Minimum	Maximum
25	German	44.5	3.6	14.5	20	72
	Polish	35.7	2.6	11.2	10	50
	Taiwanese	24.9	3.5	15.0	2	48
50	German	26.3	3.5	15.7	2	60

Dpi	Parasite population	Mean	SE	SD	Minimum	Maximum
	Polish	20.0	3.7	14.7	0	50
	Taiwanese	24.8	5.2	22.5	0	80
100	German	8.7	2.9	12.0	0	40
	Polish	11.1	2.4	9.7	0	32
	Taiwanese	10.0	2.3	10.3	0	38
150	German	6.4	1.4	5.9	0	20
	Polish	5.3	1.0	4.4	0	14
	Taiwanese	4.0	1.4	6.1	0	26

In order to check for differences in the number of worms recovered between populations of *A. crassus* from Germany, Poland and Taiwan Mann-Whitney U-tests and fixed-effects linear models were computed.

3.2.1.1.1 Non-parametric Mann Whitney U-tests

The Mann-Whitney U-tests revealed no differences in recovery between the Polish and German populations of *A. crassus* in neither the European nor in the Japanese eel hosts. However, significant differences between the European and the Taiwanese parasite populations could be shown in both eel species, suggesting altered infectivity of the different parasite populations when harboured in the same eel species (Table 11; Figure 20).

The differences in recovery were more pronounced in the European than in the Japanese eel. In the European eel the recovery of the Taiwanese worms was constantly higher during the whole infection period (Table 9, 11; Figure 20). In the Japanese eel the percentage of recovered Taiwanese nematodes was lower at 25 dpi only, while for all other investigation intervals no significant differences between the populations could be found (Tables 10, 11; Figure 20).

Table 11: P-values of Mann-Whitney U-tests for pair wise comparisons of recovery between populations of *Anguillicola crassus* from Germany, Poland and Taiwan in *Anguilla anguilla* and *Anguilla japonica*. Dpi – days post infection.

		Anguilla anguilla			***Anguilla japonica***		
Stage	dpi	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan
Recovery	25	0.329	0.025	0.030	0.092	0.029	0.001
	50	0.303	0.013	0.000	0.231	0.787	0.499

		Anguilla anguilla			*Anguilla japonica*		
	100	0.194	0.000	0.000	0.205	0.655	0.415
	150	0.080	0.000	0.000	0.780	0.146	0.08

Higher value: — Poland — Germany — Taiwan

3.2.1.1.2 Fixed-effects linear models

Similarly to the U-tests, the Taiwan-Germany-Poland fixed-effects linear models revealed no significant differences in recovery between the investigated populations of *A. crassus* from Germany and Poland (Boxes 1 - 2).

Box 1: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 1: Recovery; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	**Estimate**	**SE**	**t-value**	**p-value**
(Intercept)	0.272855	0.020805	13.115	**0.0000**
Japanese eel	0.106723	0.026852	3.974	**0.0000**
Polish parasite population	-0.006613	0.027928	-0.237	0.8129
Taiwanese parasite population	0.117831	0.027962	4.214	**0.0000**
Dpi	-0.000932	0.000262	-3.552	**0.0004**
Japanese eel*Polish parasite population	-0.050290	0.031617	-1.591	0.1124
Japanese eel*Taiwanese parasite population	-0.251314	0.031407	-8.002	**0.0000**
Japanese eel*Dpi	-0.002023	0.000268	-7.552	**0.0000**
Polish parasite population*Dpi	0.000480	0.000328	1.461	0.1446
Taiwanese parasite population*Dpi	0.001338	0.000327	4.089	**0.0000**

Box 2: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 9: Recovery; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	**Estimate**	**SE**	**t-value**	**p-value**
(Intercept)	0.266242	0.020891	12.744	**0.0000**
Japanese eel	0.056433	0.027114	2.081	**0.0380**
German parasite population	0.006613	0.027928	0.237	0.8129
Taiwanese parasite population	0.124445	0.028040	4.438	**0.0000**
Dpi	-0.000452	0.000261	-1.736	0.0832
Japanese eel*German parasite population	0.050290	0.031617	1.591	0.1124
Japanese eel*Taiwanese parasite population	-0.201025	0.031499	-6.382	**0.0000**
Japanese eel*Dpi	-0.002023	0.000268	-7.552	**0.0000**

Explanatory variables and interactions	Estimate	SE	t-value	p-value
German parasite population*Dpi	-0.000479	0.000328	-1.461	0.1447
Taiwanese parasite population*Dpi	0.000859	0.000326	2.631	**0.0088**

As both European populations were similar in terms of recovery they were pooled into one group (European parasite populations) and a further linear model was calculated (Box 3). The model revealed differences between the pooled European parasite populations and the Taiwanese population. In the European eel the Taiwanese nematodes had the highest recovery, which decreased more slowly than in all other host-parasite combinations (Box 3; Figure 20). In the Japanese eel the Taiwanese parasite population had a lower recovery than the European populations (Box 3). However, its slower decrease resulted in similar recoveries at later infection periods (Figure 20).

Box 3: Minimal adequate fixed-effects linear model (Taiwan-Europe) 17: Recovery; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	0.393548	0.021674	18.158	**0.0000**
Japanese eel	-0.173514	0.027791	-6.244	**0.0000**
European parasite populations	-0.124418	0.024995	-4.978	**0.0000**
Dpi	0.000510	0.000278	1.833	0.0676
Japanese eel*European parasite populations	0.240831	0.028534	8.440	**0.0000**
Japanese eel*Dpi	-0.001928	0.000283	-6.806	**0.0000**
European parasite populations*Dpi	-0.001186	0.000297	-3.993	**0.0000**

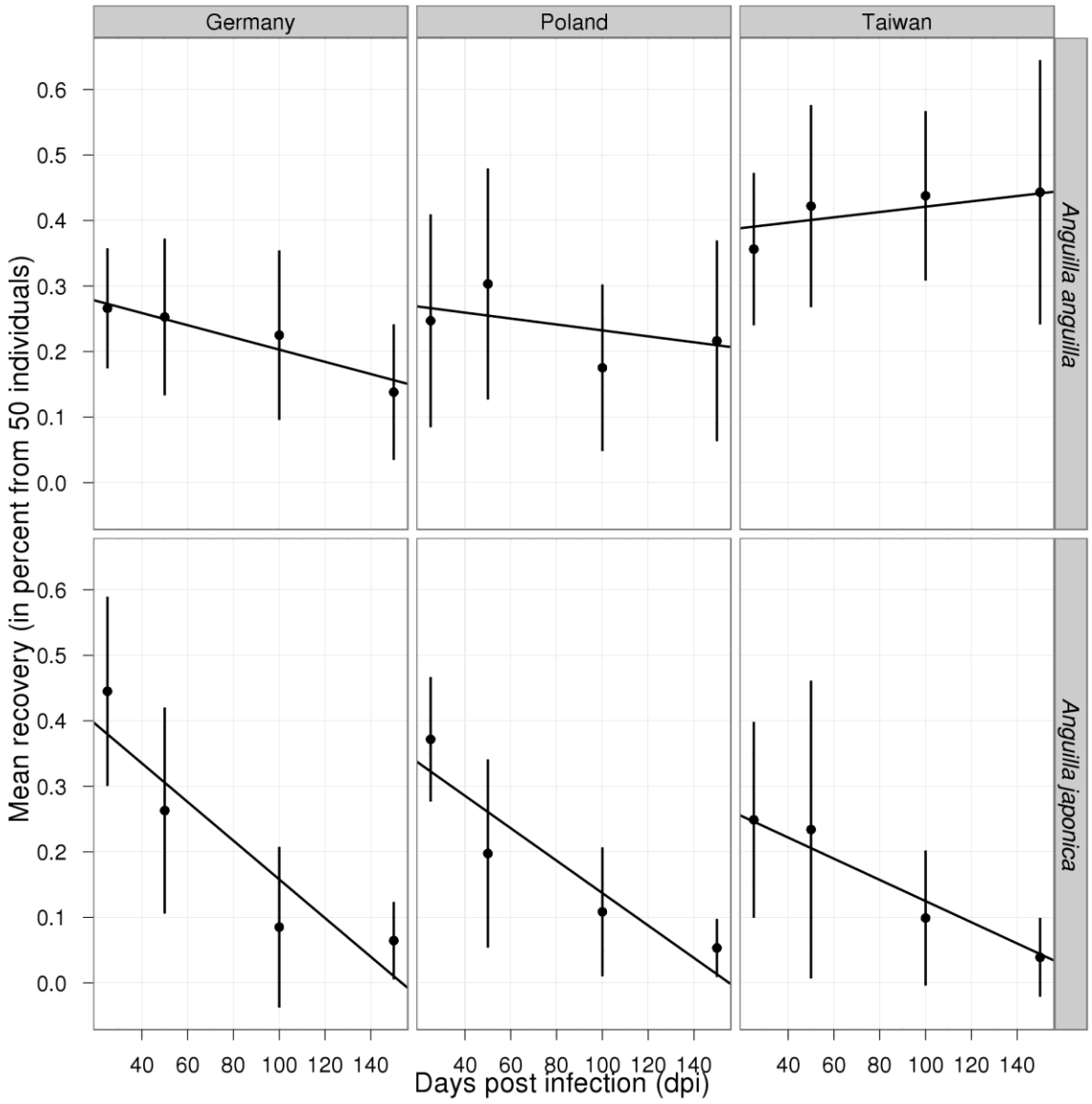

Figure 17: Recovery of *Anguillicola crassus* populations from Germany, Poland and Taiwan in *Anguilla anguilla* and *Anguilla japonica* – mean values with standard deviation and correction curves fitted in the linear models.

3.2.1.2 Differences in the developmental dynamics

Developmental dynamics refers to the speed of moulting to the next developmental stage (L3 → L4 (+ dead larvae) → adults → dead adults) and was estimated by comparisons of the mean number of each life history stage found at each dpi. The mean, standard error (SD), standard deviation (SE) and minimum and maximum values of the numbers of the developmental stages of *A. crassus* in the European and Japanese eel are presented in tables 12 and 13. Again a high overdispersion of the data could be observed, potentially indicating a negative binomial distribution among the life history stages in different eel individuals.

Table 12: The mean numbers of life history stages of *Anguillicola crassus* from Germany, Poland and Taiwan in the European eel with standard error (SE), standard deviation (SD) and minimum and maximum values; D pi - days post infection.

Dpi	Parasite population	Life history stage	Mean	SE	SD	Minimum	Maximum
25	German	L3	7.0	1.0	4.3	0	15
		L4	5.8	0.5	2.2	1	9
		Adults recovered alive	0.6	0.2	0.8	0	2
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.0	0.0	0.0	0	0
	Polish	L3	6.5	1.2	5.2	0	15
		L4	5.6	1.0	4.5	0	14
		Adults recovered alive	0.3	0.1	0.7	0	2
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.1	0.1	0.2	0	1
	Taiwanese	L3	15.7	1.1	5.4	5	24
		L4	2.1	0.4	1.9	0	6
		Adults recovered alive	0.0	0.0	0.0	0	0
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.0	0.0	0.2	0	1
50	German	L3	3.2	0.6	2.9	0	10
		L4	2.3	0.4	2.1	0	10
		Adults recovered alive	7.1	0.5	2.6	1	13
		Dead adults	0.7	0.4	1.8	0	8
		Dead larvae	0.1	0.1	0.3	0	1
	Polish	L3	6.0	1.2	6.1	0	25
		L4	2.4	0.3	1.4	0	5
		Adults recovered alive	6.7	0.8	3.9	0	20
		Dead adults	0.4	0.1	0.7	0	3
		Dead larvae	0.1	0.1	0.3	0	1
	Taiwanese	L3	14.0	1.6	7.2	0	27
		L4	2.3	0.2	1.1	1	4
		Adults recovered alive	4.8	0.8	3.5	0	15
		Dead adults	0.1	0.1	0.2	0	1
		Dead larvae	0.0	0.0	0.0	0	0
100	German	L3	2.2	0.5	2.2	0	7
		L4	1.2	0.4	1.6	0	5
		Adults recovered alive	7.9	0.9	4.2	1	18
		Dead adults	0.5	0.2	0.8	0	3
		Dead larvae	0.4	0.1	0.6	0	2
	Polish	L3	1.5	0.6	2.4	0	9
		L4	1.0	0.3	1.3	0	4
		Adults recovered alive	6.3	1.2	4.8	1	21
		Dead adults	0.9	0.3	1.4	0	5
		Dead larvae	0.1	0.1	0.3	0	1

Dpi	Parasite population	Life history stage	Mean	SE	SD	Minimum	Maximum
	Taiwanese	L3	12.7	1.3	5.5	0	23
		L4	1.7	0.3	1.3	0	5
		Adults recovered alive	7.6	0.8	3.4	4	15
		Dead adults	0.8	0.4	1.7	0	6
		Dead larvae	0.2	0.1	0.4	0	1
150	German	L3	0.9	0.3	1.6	0	4
		L4	0.8	0.3	1.6	0	6
		Adults recovered alive	5.2	0.8	3.9	0	12
		Dead adults	2.4	0.6	2.6	0	8
		Dead larvae	0.6	0.2	0.9	0	3
	Polish	L3	2.2	0.6	2.8	0	8
		L4	1.3	0.3	1.5	0	4
		Adults recovered alive	7.4	1.1	5.3	0	18
		Dead adults	1.5	0.3	1.5	0	5
		Dead larvae	0.3	0.1	0.6	0	2
	Taiwanese	L3	12.1	1.3	5.8	3	25
		L4	2.3	0.3	1.5	0	5
		Adults recovered alive	7.8	1.3	5.5	0	16
		Dead adults	0.6	0.3	1.1	0	4
		Dead larvae	0.2	0.1	0.4	0	1

Table 13: The mean numbers of life history stages of *Anguillicola crassus* from Germany, Poland and Taiwan in the Japanese eel with standard error (SE), standard deviation (SD) and minimum and maximum values; Dpi - days post infection.

Dpi	Parasite population	Life history stage	Mean	SE	SD	Minimum	Maximum
25	German	L3	8.4	1.3	5.3	1	19
		L4	8.9	0.6	2.5	3	13
		Adults recovered alive	4.9	1.0	4.1	0	14
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.1	0.1	0.3	0	1
	Polish	L3	7.9	1.4	5.8	0	25
		L4	7.1	0.8	3.5	0	15
		Adults recovered alive	3.1	0.8	3.2	0	9
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.0	0.0	0.0	0	0
	Taiwanese	L3	3.7	1.0	4.3	0	17
		L4	8.3	1.4	5.8	0	22
		Adults recovered alive	0.4	0.3	1.1	0	4
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	0.1	0.1	0.3	0	1
50	German	L3	4.9	1.2	5.3	0	16

Dpi	Parasite population	Life history stage	Mean	SE	SD	Minimum	Maximum
		L4	3.2	0.5	2.4	0	8
		Adults recovered alive	5.1	0.8	3.5	0	11
		Dead adults	0.1	0.1	0.2	0	1
		Dead larvae	8.4	1.3	5.9	0	18
	Polish	L3	3.9	1.1	4.5	0	16
		L4	3.0	0.6	2.3	0	8
		Adults recovered alive	3.0	0.8	3.4	0	12
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	6.5	0.7	2.9	2	11
	Taiwanese	L3	3.8	1.0	4.5	0	13
		L4	3.6	0.8	3.6	0	13
		Adults recovered alive	4.4	1.2	5.3	0	20
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	2.3	1.0	4.5	0	14
100	German	L3	1.8	1.1	4.5	0	18
		L4	1.3	0.5	1.9	0	7
		Adults recovered alive	1.4	0.4	1.6	0	5
		Dead adults	0.1	0.1	0.3	0	1
		Dead larvae	11.0	1.4	5.7	1	21
	Polish	L3	0.8	0.3	1.2	0	3
		L4	2.0	0.5	2.3	0	7
		Adults recovered alive	2.3	0.6	2.4	0	10
		Dead adults	0.1	0.1	0.5	0	2
		Dead larvae	8.7	1.3	5.7	0	19
	Taiwanese	L3	1.0	0.4	1.8	0	6
		L4	1.8	0.5	2.0	0	7
		Adults recovered alive	2.2	0.6	2.6	0	11
		Dead adults	0.1	0.1	0.2	0	1
		Dead larvae	10.3	1.5	6.6	0	30
150	German	L3	0.3	0.3	1.4	0	6
		L4	0.6	0.2	1.0	0	3
		Adults recovered alive	2.3	0.5	2.2	0	7
		Dead adults	0.3	0.2	0.7	0	2
		Dead larvae	13.2	1.9	8.1	4	33
	Polish	L3	0.1	0.1	0.3	0	1
		L4	0.6	0.3	1.1	0	4
		Adults recovered alive	2.0	0.5	2.1	0	7
		Dead adults	0.3	0.2	0.7	0	2
		Dead larvae	14.6	1.8	7.7	4	33
	Taiwanese	L3	0.3	0.1	0.6	0	2
		L4	1.0	0.4	1.6	0	6
		Adults recovered alive	0.8	0.3	1.3	0	5
		Dead adults	0.0	0.0	0.0	0	0
		Dead larvae	13.6	1.8	8.1	1	32

In order to check for differences in developmental dynamics between populations of *A. crassus* from Germany, Poland and Taiwan the numbers of life history stages at each infection period (U - tests) and during the whole experiment (fixed-effects linear models) were statistically compared.

3.2.1.2.1 Non-parametric Mann-Whitney U-tests

The Mann-Whitney U-tests revealed differences in developmental dynamics between the European and the Taiwanese parasite populations in both eel species, suggesting faster development of the European worms (Table 14; Figure 21). Similarly to recovery, these differences were more pronounced in the European than in the Japanese eel. The European populations of *A. crassus* from Germany and Poland showed no significant differences from one another (Table 14).

Table 14: P-values of Mann-Whitney U-tests for pair wise comparisons of the numbers of developmental stages between populations of *Anguillicola crassus* from Germany, Poland and Taiwan in *Anguilla anguilla* and *Anguilla japonica*. Dpi – days post infection.

		Anguilla anguilla			***Anguilla japonica***		
Stage	dpi	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan
L3	25	0.663	0.000	0.000	0.752	0.003	0.004
	50	0.177	0.000	0.000	0.854	0.784	0.540
	100	0.238	0.000	0.000	0.925	0.838	0.839
	150	0.063	0.000	0.000	1.000	0.574	0.344
L4	25	0.165	0.009	0.000	0.061	0.504	0.555
	50	0.284	0.795	0.308	0.942	0.811	0.994
	100	0.999	0.060	0.095	0.514	0.817	0.417
	150	0.222	0.037	0.001	1.000	0.428	0.468
Adults	25	0.268	0.048	0.001	0.139	0.005	0.000
	50	0.446	0.041	0.005	0.080	0.469	0.241
	100	0.157	0.286	0.687	0.067	0.523	0.246
	150	0.184	0.851	0.107	0.640	0.021	0.006
Dead	25	1.000	1.000	1.000	1.000	1.000	1.000

		Anguilla anguilla			*Anguilla japonica*		
adults	50	0.726	0.020	0.042	1.000	1.000	1.000
	100	0.934	0.265	0.474	0.437	0.934	0.584
	150	0.174	0.032	0.003	0.907	0.218	0.800
Dead larvae	25	1.000	1.000	1.000	0.227	0.486	1.000
	50	0.463	0.027	0.242	0.481	0.000	0.000
	100	0.118	1.000	0.118	0.437	0.910	0.511
	150	0.568	0.603	0.149	0.485	0.800	0.778

Higher value — Poland — Germany — Taiwan

Differences in the European eel

The L3 larvae from the Taiwanese population were represented in higher numbers during the whole experiment and the numbers of L4 larvae were lower at 25 but higher at 150 dpi (Tables 12, 14; Figure 20). Adult worms from the Taiwanese population showed lower intensities at 25 and 50 dpi and dead adults showed lower intensities at 50 and 150 dpi (Tables 12, 14; Figure 20). This indicates that the most worms from the Taiwanese population were in the L3 larval stage during the whole infection period, while worms belonging to the European populations had already developed to the L4 or adult stages. The higher number of dead adults belonging to the European populations beginning from 50 dpi suggests that these nematodes completed their developmental cycle earlier than their Taiwanese conspecifics. The only difference in the numbers of dead larvae in this eel species was found between the Polish and Taiwanese population, with a higher proportion of dead larvae in the Polish population at 50 dpi (Tables 12, 14).

Differences in the Japanese eel

The numbers of L3 larvae from the Taiwanese population were lower at 25 dpi (Tables 13, 14; Figure 20). However, the numbers of adult worms were also lower at 25 and 150 dpi, again suggesting faster development from larvae to adults of the Taiwanese worms. Additionally, in this eel species the numbers of Taiwanese dead larvae were lower at 50 dpi (Tables 13, 14; Figure 20).

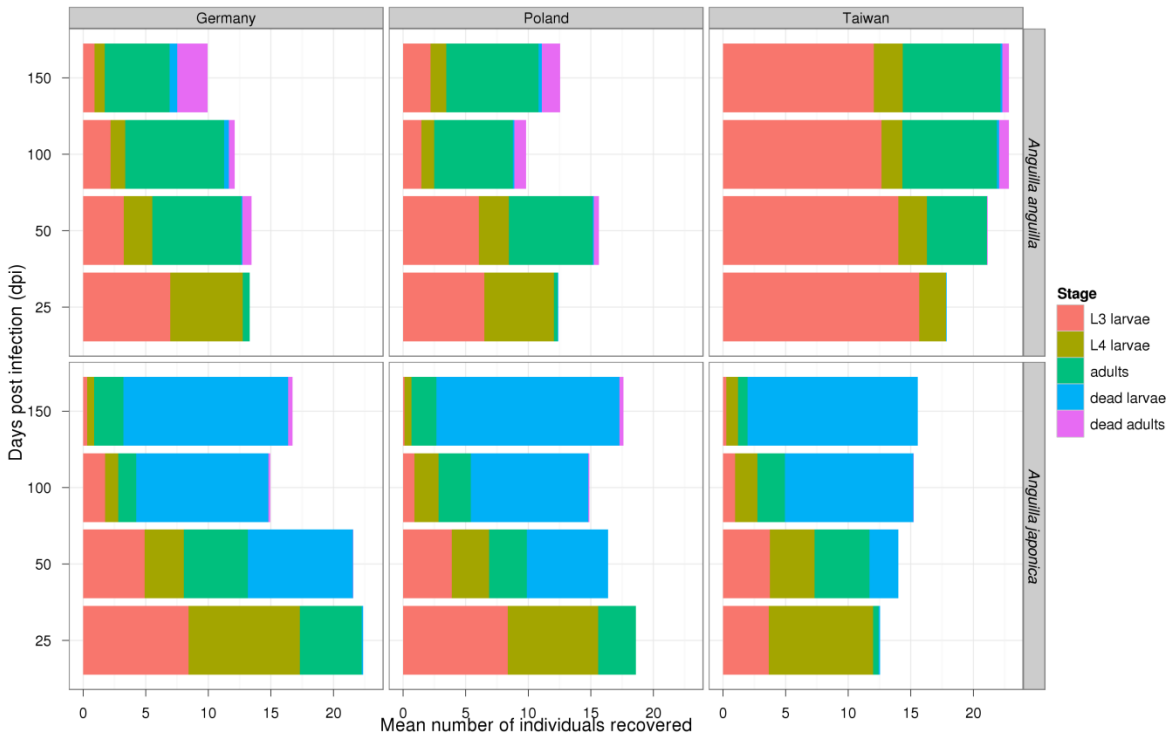

Figure 18: Mean number of the life history stages of *Anguillicola crassus* populations in *Anguilla anguilla* and *Anguilla japonica*.

3.2.1.2.2 Fixed-effects linear models

The Taiwan-Germany-Poland fixed-effects linear models revealed no significant differences in numbers of the developmental stages between the investigated populations of *A. crassus* from Germany and Poland (Boxes 4 - 13).

Box 4: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 2: L3; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	3.985710	0.654633	6.088	**0.0000**
Japanese eel	0.083804	0.734940	0.114	0.9093
Polish parasite population	0.854633	0.661333	1.292	0.1970
Taiwanese parasite population	10.665572	0.687459	15.514	**0.0000**
Dpi	-0.023870	0.005617	-4.250	**0.0000**
Number of L4 recovered alive	0.371018	0.075102	4.940	**0.0000**
Mean length of adults	-0.080773	0.030669	-2.634	**0.0088**
Number of adults recovered alive	0.170534	0.063373	2.691	**0.0074**
Japanese eel*Polish parasite population	-1.456384	1.014095	-1.436	0.1517
Japanese eel*Taiwanese parasite population	-12.606443	1.009295	-12.490	**0.0000**

Box 5: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 3: L4; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	3.530272	0.466087	7.574	**0.0000**
Japanese eel	1.661341	0.681925	2.436	**0.0153**
Polish parasite population	-0.215684	0.614358	-0.351	0.7257
Taiwanese parasite population	-3.673322	0.687534	-5.343	**0.0000**
Dpi	-0.028014	0.006113	-4.582	**0.0000**
Number of L3 recovered alive	0.144385	0.029194	4.946	**0.0000**
Japanese eel*Polish parasite population	-0.334658	0.964588	-0.347	0.7288
Japanese eel*Taiwanese parasite population	4.078635	1.021768	3.992	**0.0000**
Japanese eel*Dpi	-0.017740	0.009301	-1.907	0.0572
Polish parasite population*Dpi	0.004663	0.008468	0.551	0.5821
Taiwanese parasite population*Dpi	0.031737	0.008742	3.630	**0.0004**
Japanese eel*Polish parasite population*Dpi	0.005799	0.013152	0.441	0.6595
Japanese eel*Taiwanese parasite population*Dpi	-0.029587	0.012948	-2.285	**0.0228**

Box 6: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 4: Adults recovered alive; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	4.184e+00	5.105e-01	8.196	**0.0000**
Japanese eel	5.021e-02	5.350e-01	0.094	0.9253

Explanatory variables and interactions	Estimate	SE	t-value	p-value
Polish parasite population	-1.400e+00	6.515e-01	-2.149	**0.0322**
Taiwanese parasite population	-2.525e+00	6.434e-01	-3.924	**0.0001**
Dpi	2.152e-02	7.927e-03	2.714	**0.0069**
Number of dead adults	-2.199e-01	2.274e-01	-0.967	0.3341
Number of eggs	2.624e-06	1.203e-06	2.181	**0.0298**
Japanese eel*Dpi	-4.839e-02	8.007e-03	-6.044	**0.0000**
Polish parasite population*Dpi	1.717e-02	9.456e-03	1.816	0.0700
Taiwanese parasite population*Dpi	2.357e-02	9.171e-03	2.570	**0.0105**
Polish parasite population* Number of dead adults	1.086e-01	4.223e-01	0.257	0.7971
Taiwanese parasite population* Number of dead adults	1.712e+00	4.557e-01	3.756	**0.0002**
Japanese eel* Number of eggs	3.869e-05	1.051e-05	3.682	**0.0003**

Box 7: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 5: Dead adults; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	2.298e-01	1.458e-01	1.576	0.1157
Japanese eel	-2.814e-02	1.450e-01	-0.194	0.8462
Polish parasite population	-1.540e-01	1.641e-01	-0.938	0.3486
Taiwanese parasite population	-4.111e-01	1.587e-01	-2.591	**0.0099**
Dpi	8.669e-03	1.649e-03	5.258	**0.0000**
Number of dead larvae	5.481e-01	1.496e-01	3.665	**0.0003**
Number of eggs	4.091e-07	4.565e-07	0.896	0.3707
Number of adults recovered alive	-2.185e-02	2.199e-02	-0.993	0.3211
Japanese eel*Dpi	-5.893e-03	2.542e-03 -	2.318	**0.0209**
Japanese eel* Number of dead larvae	-5.597e-01	1.500e-01	-3.732	**0.0002**
Polish parasite population* Number of eggs	-2.855e-07	6.387e-07	-0.447	0.6551
Taiwanese parasite population* Number of eggs	-2.114e-06	8.005e-07	-2.641	**0.0086**
Polish parasite population* Number of adults recovered alive	2.213e-02	2.883e-02	0.768	0.4432
Taiwanese parasite population* Number of adults recovered alive	7.455e-02	3.105e-02	2.401	**0.0168**

Box 8: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 6: Dead larvae; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	0.097275	0.508165	0.191	0.8483
Japanese eel	3.185007	0.778692	4.090	**0.0000**

Explanatory variables and interactions	Estimate	SE	t-value	p-value
Polish parasite population	-0.120702	0.597017	-0.202	0.8399
Taiwanese parasite population	-0.151214	0.621578	-0.243	0.8079
Dpi	0.002331	0.005567	0.419	0.6757
Number of dead adults	0.060072	0.197617	0.304	0.7613
Japanese eel*Polish parasite population	-1.084099	0.913944	-1.186	0.2362
Japanese eel*Taiwanese parasite population	-2.635698	0.913796	-2.884	**0.0041**
Japanese eel*Dpi	0.103430	0.008162	12.672	**0.0000**
Japanese eel* Number of dead adults	-2.563565	0.829422	-3.091	**0.0021**

Box 9: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 10: L3; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	4.840344	0.669593	7.229	**0.0000**
Japanese eel	-1.372580	0.747930	-1.835	0.0672
German parasite population	-0.854633	0.661333	-1.292	0.1970
Taiwanese parasite population	9.810938	0.697750	14.061	**0.0000**
Dpi	-0.023870	0.005617	-4.250	**0.0000**
Number of L4 recovered alive	0.371018	0.075102	4.940	**0.0000**
Mean length of adults	-0.080773	0.030669	-2.634	**0.0088**
Number of adults recovered alive	0.170534	0.063373	2.691	**0.007**4
Japanese eel*German parasite population	1.456384	1.014095	1.436	0.1517
Japanese eel*Taiwanese parasite population	-11.150059	1.013449	-11.002	**0.0000**

Box 10: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 11: L4; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	3.314589	0.469547	7.059	**0.0000**
Japanese eel	1.326683	0.682662	1.943	0.0526
German parasite population	0.215684	0.614358	0.351	0.7257
Taiwanese parasite population	-3.457639	0.676078	-5.114	**0.0000**
Dpi	-0.023350	0.006083	-3.838	**0.0001**
Number of L3 recovered alive	0.144385	0.029194	4.946	**0.0000**
Japanese eel*German parasite population	0.334658	0.964588	0.347	0.7288
Japanese eel*Taiwanese parasite population	4.413292	1.008075	4.378	**0.0000**
Japanese eel*Dpi	-0.011941	0.009339	-1.279	0.2018

Explanatory variables and interactions	Estimate	SE	t-value	p-value
German parasite population*Dpi	-0.004663	0.008468	-0.551	0.5821
Taiwanese parasite population*Dpi	0.027073	0.008729	3.102	**0.0020**
Japanese eel*German parasite population*Dpi	-0.005799	0.013152	-0.441	0.6595
Japanese eel*Taiwanese parasite population*Dpi	-0.035386	0.012975	-2.727	**0.0067**

Box 11: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 12: Adults recovered alive; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	2.784e+00	5.083e-01	5.476	**0.0000**
Japanese eel	5.021e-02	5.350e-01	0.094	0.9253
German parasite population	1.400e+00	6.515e-01	2.149	**0.0322**
Taiwanese parasite population	-1.125e+00	6.434e-01	-1.748	0.0812
Dpi	3.869e-02	8.090e-03	4.783	**0.0000**
Number of dead adults	-1.113e-01	3.670e-01	-0.303	0.7618
Number of eggs	2.624e-06	1.203e-06	2.181	**0.0298**
Japanese eel*Dpi	-4.839e-02	8.007e-03	-6.044	**0.0000**
German parasite population*Dpi	-1.717e-02	9.456e-03	-1.816	0.0700
Taiwanese parasite population*Dpi	6.396e-03	9.230e-03	0.693	0.4888
German parasite population* Number of dead adults	-1.086e-01	4.223e-01	-0.257	0.7971
Taiwanese parasite population* Number of dead adults	1.603e+00	5.337e-01	3.004	**0.002**8
Japanese eel* Number of eggs	3.869e-05	1.051e-05	3.682	**0.0003**

Box 12: Minimal adequate fixed-effects linear model (Taiwan - Germany - Poland): Dead adults; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	7.584e-02	1.332e-01	0.569	0.5694
Japanese eel	-2.814e-02	1.450e-01	-0.194	0.8462
German parasite population	1.540e-01	1.641e-01	0.938	0.3486
Taiwanese parasite population	-2.571e-01	1.491e-01	-1.724	0.0855
Dpi	8.669e-03	1.649e-03	5.258	**0.000**0
Number of dead larvae	5.481e-01	1.496e-01	3.665	**0.000**3
Number of eggs	1.236e-07	4.945e-07	0.250	0.8028
Number of adults recovered alive	2.790e-04	1.947e-02	0.014	0.9886
Japanese eel*Dpi	-5.893e-03	2.542e-03	-2.318	**0.0209**
Japanese eel* Number of dead larvae	-5.597e-01	1.500e-01	-3.732	**0.0002**
German parasite population* Number of	2.855e-07	6.387e-07	0.447	0.6551

Explanatory variables and interactions	Estimate	SE	t-value	p-value
eggs				
Taiwanese parasite population* Number of eggs	-1.829e-06	8.140e-07	-2.247	**0.0252**
German parasite population* Number of adults recovered alive	-2.213e-02	2.883e-02	-0.768	0.4432
Taiwanese parasite population* Number of adults recovered alive	5.242e-02	2.900e-02	1.808	**0.071**4

Box 13: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 14: Dead larvae; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-0.023427	0.508669	-0.046	0.9633
Japanese eel	2.100909	0.781057	2.690	**0.0074**
German parasite population	0.120702	0.597017	0.202	0.8399
Taiwanese parasite population	-0.030512	0.624215	-0.049	0.9610
Dpi	0.002331	0.005567	0.419	0.6757
Number of dead adults	0.060072	0.197617	0.304	0.7613
Japanese eel*German parasite population	1.084099	0.913944	1.186	0.2362
Japanese eel*Taiwanese parasite population	-1.551599	0.915491	-1.695	0.0909
Japanese eel*Dpi	0.103430	0.008162	12.672	**0.0000**
Japanese eel* Number of dead adults	-2.563565	0.829422	-3.091	**0.0021**

Thus, both European populations were pooled and further fixed-effects linear models were applied (Boxes 14 - 18). These models displayed marked differences in developmental dynamics between the Taiwanese and European parasite populations in the European eel. In the Japanese eel most patterns were similar for all parasite populations, although some important differences were also present between European and Taiwanese worms.

Box 14: Minimal adequate fixed-effects linear model (Taiwan-Europe) 18: L3; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	14.639495	0.643787	22.740	**0.0000**
Japanese eel	-12.516307	0.750519	-16.677	**0.0000**
European parasite populations	-10.251415	0.608706	-16.841	**0.0000**
Dpi	-0.023961	0.005619	-4.265	**0.0000**
Number of L4 recovered alive	0.372899	0.075113	4.965	**0.0000**
Mean length of adults	-0.080428	0.030657	-2.623	**0.0090**

Explanatory variables and interactions	Estimate	SE	t-value	p-value
Number of adults recovered alive	0.172357	0.063274	2.724	**0.0067**
Japanese eel*European parasite populations	11.888387	0.875332	13.582	**0.0000**

Box 15: Minimal adequate fixed-effects linear model (Taiwan-Europe) 19: L4; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-0.145651	0.632174	-0.230	0.8179
Japanese eel	5.741970	0.735904	7.803	**0.0000**
European parasite populations	3.564495	0.606427	5.878	**0.0000**
Dpi	0.003728	0.006378	0.584	0.5592
Number of L3 recovered alive	0.144553	0.029013	4.982	**0.0000**
Japanese eel*European parasite populations	-4.241377	0.889797	-4.767	**0.0000**
Japanese eel*Dpi	-0.047327	0.008982	-5.269	**0.0000**
European parasite populations*Dpi	-0.029405	0.007616	-3.861	**0.0001**
Japanese eel*European parasite populations*Dpi	0.032458	0.011133	2.915	**0.0037**

Box 16: Minimal adequate fixed-effects linear model (Taiwan-Europe) 20: Adults recovered alive; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	2.027e+00	4.377e-01	4.631	**0.0000**
Japanese eel	-1.175e-02	5.333e-01	-0.022	0.9824
European parasite populations	1.334e+00	3.959e-01	3.370	**0.0008**
Dpi	3.346e-02	5.916e-03	5.655	**0.0000**
Number of dead adults	1.518e+00	4.002e-01	3.794	**0.0002**
Number of eggs	7.873e-06	2.324e-06	3.387	**0.0008**
Japanese eel*Dpi	-4.596e-02	8.000e-03	-5.745	**0.0000**
European parasite populations*Number of dead adults	-1.715e+00	4.324e-01	-3.966	**0.0000**
Japanese eel* Number of eggs	3.800e-05	1.042e-05	3.649	**0.0003**
European parasite populations* Number of eggs	-6.456e-06	2.471e-06	-2.613	**0.0093**

Box 17: Minimal adequate fixed-effects linear model (Taiwan-Europe) 21: Dead adults; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-1.819e-01	1.284e-01	-1.417	0.1574
Japanese eel	-3.224e-02	1.446e-01	-0.223	0.8237
European parasite populations	3.247e-01	1.296e-01	2.506	**0.0126**
Dpi	8.719e-03	1.631e-03	5.347	**0.0000**
Number of dead larvae	5.464e-01	1.455e-01	3.754	**0.0002**
Number of eggs	-1.711e-06	6.873e-07	-2.489	**0.0132**
Number of adults recovered alive	5.252e-02	2.266e-02	2.318	**0.0209**
Japanese eel*Dpi	-5.914e-03	2.530e-03	-2.338	**0.0199**
Japanese eel* Number of dead larvae	-5.575e-01	1.460e-01	-3.819	**0.0001**
European parasite populations* Number of eggs	1.974e-06	7.396e-07	2.669	**0.0079**
European parasite populations* Number of adults recovered alive	-6.173e-02	2.619e-02	-2.357	**0.0189**

Box 18: Minimal adequate fixed-effects linear model (Taiwan-Europe) 22: Dead larvae; reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-0.054408	0.525326	-0.104	0.9176
Japanese eel	0.554007	0.770304	0.719	0.4724
European parasite populations	0.091419	0.547412	0.167	0.8674
Dpi	0.002322	0.005575	0.417	0.6772
Number of dead adults	0.062589	0.197479	0.317	0.7514
Japanese eel*European parasite populations	2.092859	0.793325	2.638	**0.0086**
Japanese eel*Dpi	0.103364	0.008172	12.648	**0.0000**
Japanese eel* Number of dead adults	-2.563784	0.830398	-3.087	**0.0021**

Differences in the Euorpean eel

Similarly to the U-tests, the models suggest faster development of the European populations. The major difference in the developmental dynamics between populations in the European eel was related to the persistently higher levels of L3 larvae for the Taiwanese population, while these were never as high in the European populations (Figure 21; Box 14). While in the Taiwanese population there were less L4 larvae and the levels of this larval stage were stable during the infection, in the European populations the numbers of L4 decreased with time (Figure 21; Box 15). Less living adults and less dead adult worms were noted for the Taiwanese population (Figure 21; Boxes 16 - 17). The number of dead larvae did not differ between populations (Box 18).

Differences in the Japanese eel

In the Japanese eel fewer L3 (Box 14), more L4 larvae (Box 15), fewer adults (Box 16) and dead adults (Box 17) were observed in the Taiwanese population (Figure 21) indicating slower development of this population in its natural host (based on the lower number of adults and dead adults). In this eel species the number of Taiwanese encapsulated larvae was lower than the number of encapsulated larvae belonging to the European populations (Figure 18; Box 22).

3.2.1.3 Differences in reproductive potential

The developmental potential of the parasite populations was estimated by counting the eggs found in the swim bladders of the eels. The mean, standard error (SE), standard deviation (SD) and minimum and maximum values of the numbers of eggs deposited by the females of *A. crassus* are presented in tables 15 and 16. The high standard deviations around the mean as well as the minimum and maximum values of the number of eggs suggest a very high overdispersion of the data indicating that the amount of eggs in different eel individuals ranged from zero to more than a thousand.

Table 15: The mean numbers of eggs of *A. crassus* from Germany, Poland and Taiwan in the European eel with standard error (SE), standard deviation (SD) and minimum and maximum values; Dpi - days post infection.

Dpi	Parasite population	Mean	SE	SD	Minimum	Maximum
25	German	0.0	0.0	0.0	0	0
	Polish	0.0	0.0	0.0	0	0
	Taiwanese	0.0	0.0	0.0	0	0
50	German	1631.2	656.2	3280.8	0	13,140
	Polish	3438.7	1556.6	7783.1	0	34,200
	Taiwanese	885.6	665.7	2977.0	0	13,140
100	German	257383.0	601,57.116	269030.8	0	972,900
	Polish	226732.2	66994.5	276225.4	0	1,095,300
	Taiwanese	76339.1	25513.8	105196.0	0	415,800
150	German	216476.2	73911.7	338705.9	0	1,199,700
	Polish	250894.6	61832.3	290019.1	0	956,500
	Taiwanese	215528.0	68128.2	304678.8	0	928,800

Table 16: The mean numbers of eggs of *Anguillicola crassus* from Germany, Poland and Taiwan in the Japanese eel with standard error (SE), standard deviation (SD) and minimum and maximum values; Dpi - days post infection.

Dpi	Parasite population	Mean	SE	SD	Minimum	Maximum
25	German	0.0	0.0	0.0	0	0
	Polish	0.0	0.0	0.0	0	0
	Taiwanese	0.0	0.0	0.0	0	0
50	German	6021.0	4212.6	18839.5	0	78,480
	Polish	4225.6	3724.7	14898.8	0	59,590
	Taiwanese	0.0	0.0	0.0	0	0
100	German	10042.8	5051.0	20825.9	0	71,060
	Polish	26280.0	15574.4	64214.7	0	198,000
	Taiwanese	9698.4	5782.1	25858.4	0	101,700
150	German	8035.9	4360.5	18500.1	0	71,220
	Polish	7084.0	5988.4	25406.8	0	107,100
	Taiwanese	297.0	297.0	1328.2	0	5,940

To check for differences in egg output between populations of the nematode from Germany, Poland and Taiwan Mann-Whitney U-tests and fixed-effects linear models were conducted.

3.2.1.3.1 Mann-Whitney U-tests

The U - tests revealed only a few differences in terms of reproductive potential between the parasite populations from Germany, Poland and Taiwan, suggesting earlier reproduction of the European populations. In the European eel at 100 dpi the Taiwanese parasite population laid lower numbers of eggs than both European parasite populations (Tables 15, 17; Figure 22). In the Japanese host the only difference in the numbers of eggs could be detected between the German and Taiwanese populations, with a lower rate of the eggs belonging to the Taiwanese parasite population at 50 dpi (Tables 16, 17; Figure 22).

Table 17: P-values of Mann-Whitney U-tests for pair wise comparisons of the numbers of eggs between populations of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica*. Dpi – days post infection.

		Anguilla anguilla			***Anguilla japonica***		
Stage	dpi	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan	Poland vs. Germany	Poland vs. Taiwan	Germany vs. Taiwan
Eggs	25	-	-	-	-	-	-
	50	0.552	0.109	0.33	0.462	0.202	0.047
	100	0.690	0.024	0.012	0.788	0.352	0.631
	150	0.653	0.583	0.764	0.658	0.218	0.072

Higher value: — Poland — Germany — Taiwan

3.2.1.3.2 Fixed-effects linear models

As with the Mann-Whitney U-tests, the Taiwan-Germany-Poland fixed-effects linear models did not reveal differences in terms of reproductive potential between Polish and German populations (Boxes 19 - 22).

Box 19: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 7: Eggs (big model); reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-2.148521	0.502978	-4.272	**0.0000**
Japanese eel	0.403016	0.554327	0.727	0.4677
Dpi	0.027343	0.006209	4.404	**0.0000**
Number of dead adults	0.477421	0.148334	3.219	**0.0014**
Number of adults recovered alive	0.211232	0.050926	4.148	**0.0000**
Mean length of adults	0.328912	0.023256	14.143	**0.0000**
Japanese eel*Dpi	-0.018313	0.008758	-2.091	**0.0373**

Box 20: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 8: Eggs (small model); reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	4.242014	0.586921	7.228	**0.0000**
Japanese eel	-2.089189	0.733539	-2.848	**0.0047**
Polish parasite population	-0.057879	0.577201	-0.100	0.9202
Taiwanese parasite population	-1.399453	0.577891	-2.422	**0.0160**
Dpi	0.060562	0.007742	7.822	**0.0000**
Japanese eel*Dpi	-0.058246	0.011420	-5.101	**0.0000**

Box 21: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 15: Eggs (big model); reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-2.148521	0.502978	-4.272	**0.0000**
Japanese eel	0.403016	0.554327	0.727	0.4677
Dpi	0.027343	0.006209	4.404	**0.0000**
Number of dead adults	0.477421	0.148334	3.219	**0.0014**
Number of adults recovered alive	0.211232	0.050926	4.148	**0.0000**
Mean length of adults	0.328912	0.023256	14.143	**0.0000**
Japanese eel*Dpi	-0.018313	0.008758	-2.091	**0.0373**

Box 22: Minimal adequate fixed-effects linear model (Taiwan-Germany-Poland) 15: Eggs (small model); reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	4.184134	0.592840	7.058	**0.0000**
Japanese eel	-2.089189	0.733539	-2.848	**0.0047**
German parasite population	0.057879	0.577201	0.100	0.9202
Taiwanese parasite population	-1.341573	0.581421	-2.307	**0.0216**
Dpi	0.060562	0.007742	7.822	**0.0000**
Japanese eel*Dpi	-0.058246	0.011420	-5.101	**0.0000**

As for the recovery and developmental dynamics, both European populations were pooled and further fixed-effects linear models were applied. These revealed no differences in the numbers of eggs between the European and Taiwanese parasite populations neither in *A. anguilla* nor in *A. japonica* (Boxes 23 - 24).

Box 23: Minimal adequate fixed-effects linear model (Taiwan-Europe) 23: Eggs (big model); reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	-2.239289	0.513624	-4.360	**0.0000**
Japanese eel	0.497046	0.564055	0.881	0.3789
Dpi	0.027848	0.006282	4.433	**0.0000**
Number of dead adults	0.560382	0.157009	3.569	**0.0004**
Number of adults recovered alive	0.213062	0.051612	4.128	**0.0000**
Mean length of adults	0.329030	0.023745	13.857	**0.0000**
Japanese eel*Dpi	-0.018661	0.008864	-2.105	**0.0361**

Box 24: Minimal adequate fixed-effects linear model (Taiwan-Europe) 24: Eggs (small model); reference group: Taiwanese parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SE	t-value	p-value
(Intercept)	3.15069	0.64053	4.919	**0.0000**
Japanese eel	-2.10616	0.76201	-2.764	**0.0060**
Dpi	0.06633	0.00804	8.250	**0.0000**
Japanese eel*Dpi	-0.06149	0.01201	-5.120	**0.0000**

As the significant differences in reproductive potential between the parasite populations when harboured in the same eel species revealed in the U-tests were constrained in each eel species to a single investigation period and the models displayed loss of significance,

I assumed a similarity of all parasite populations in respect to this trait.

3.2.2 Host-dependent developmental patterns

The fixed – effects – linear models additionally revealed general developmental patterns that were linked to the host species being infected. These traits were similar for all parasite populations in the same eel species, independently from their origin. The recovery in the Japanese eel was generally lower and decreased faster in the course of infection (Figure 20; Box 3). My data suggest that in the Japanese eel the decrease in recovery was associated with a pronounced decrease of L4 larval stages and adults, and a simultaneous, pronounced increase of the number of dead larvae (Figure 21; Boxes 15, 16, 18). The total number of dead worms in the native host seems to be regulated within the infrapopulation, as the number of dead adults decreased with increasing number of dead larvae (Box 17) and the number of dead larvae decreased with increasing number of dead adults (Box 18). Additionally, in the Japanese eel the number of dead adults increased more slowly, resulting in lower numbers of this developmental stage during the infection period (Box 17). In comparison, in the European eel all parasite populations showed higher recovery and this decrease in recovery was accompanied by a pronounced increase in living and dead adult worms (Figure 20; Boxes 3, 16, 17). Finally, the egg production of the parasites differed substantially depending on the eel species being infected. In the Japanese eel all of the parasite populations exhibited a lower reproductive output reflected by a slower increase in the number of eggs discharged by female adults with time (Box 23), and in lower numbers of eggs per infrapopulation (Figure 21; Box 24). Finally, in the natural host the positive influence of the eggs on the number of adults recovered alive (an aspect referring to the regulation of the infrapopulation density) was more apparent than in the colonized host (Box 16).

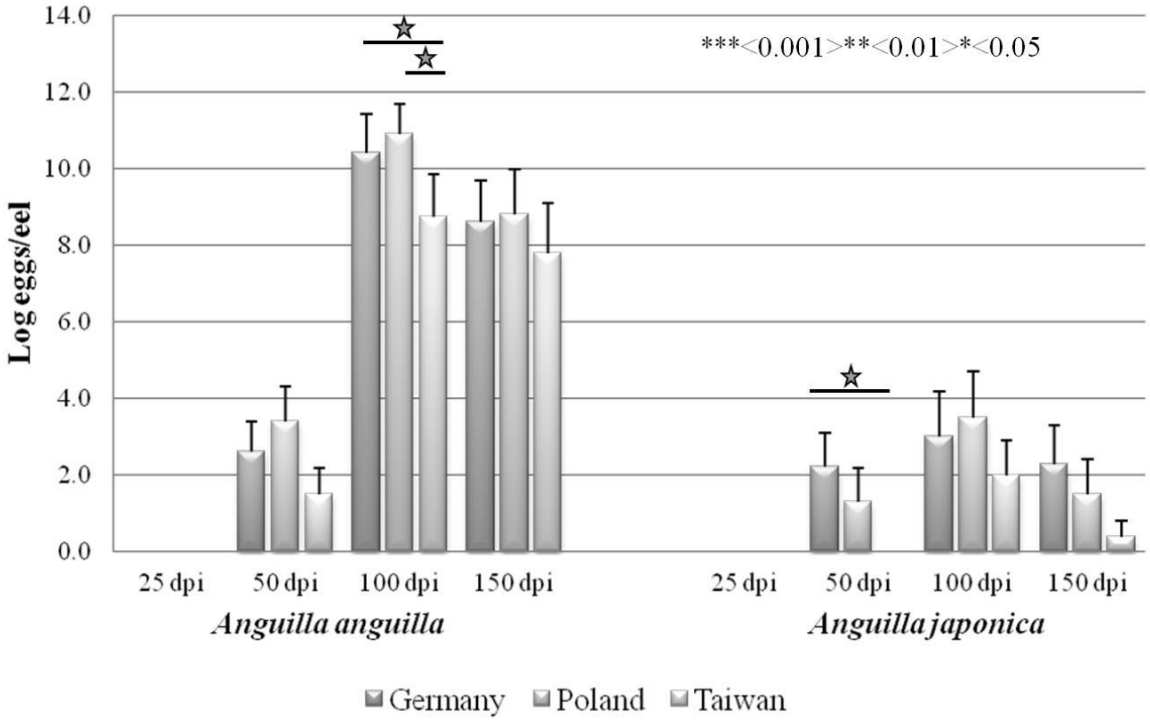

Figure 19: Log mean number of eggs of *Anguillicola crassus* per eel (*Anguilla anguilla* and *Anguilla japonica)*. An asterisk refers to the significance levels of the U - tests (see the section on differences in reproductive potential between populations of *Anguillicola crassus*, descriptive statistics and non-parametric Mann-Whitney U -tests). Dpi – days post infection.

3.2.3 Other effects: self-regulation and host-dependent regulation of infrapopulation density

The models also predicted other effects that may play a role in the density regulation process, independently of the influences of host species and parasite population. There were positive reciprocal effects of L4 larvae on the number of L3 larvae (Boxes 14, 15). Moreover, the negative impact of the length of adult worms and the positive impact of the numbers of adult worms recovered alive on the L3 density could be noted. In addition, the numbers of adult worms recovered alive should positively influence the dead adult intensity (Box 14). For both eel species I also found that the number of eggs laid per eel was positively associated with the number of adults, the number of dead adults and the length of adults (Box 23). The increased number of eggs should have a positive effect on the number of adults and the number of dead adults (Boxes 20 - 21).

However, for the Taiwanese population a positive effect of the number of dead larvae on the number of dead adults was detected (Box 20).

The only differences between the eel species in the regulatory mechanisms were conveyed in the more pronounced reciprocal negative association between the dead larvae and dead adults (Boxes 21 – 22) and a more pronounced positive influence of the eggs laid within a swim bladder on the number of adults observed in the Japanese eel (Box 20).
In all cases there was a high degree of variability within the parameter values so that R^2 was low.
The fixed-effects linear models showed no relationship between the eel sex, eel length or tank number on recovery (Box 3). In addition, there was no association between the eel length or the tank number on the numbers of developmental stages of *A. crassus* recovered (Boxes 14 - 18, 23 - 24).

3.3 Morphology of populations of *Anguillicola crassus* in the European and Japanese eel

In order to look for morphological differences between populations of *A. crassus* from Germany, Poland and Taiwan the length and width of the body, oesophagus and buccal capsule were measured. To describe differences in the shape of these traits the ratios of each measurement pair (length/width) were estimated (the smaller ratio the rounder/fatter the morphological trait). All statistics were performed separately for females and males. The separation of genders was required due to the morphometric dimorphism of the adult worms with females being larger than males (Taraschewski et al., 1987). First of all, descriptive statistics were computed. Next, mixed-effects linear models were applied in order to reveal whether morphometric differences occurred between the nematode populations during the experiment. As the number of adult worms at 25 dpi was statistically insufficient, this investigation interval was omitted in the analysis.

In the European eel a total of 1,279 adult worms were found with the sex distribution skewed towards males with 39.6% females and 60.4% males. 1,136 adult worms were chosen for the morphological investigations: 424 (45.4% female, 54.6% male) worms belonging to the German, 387 (41.4% female, 58.6% male) to the Polish and 325 (30% female, 70% male) to the Taiwanese worm population. In the Japanese eels a total of 585 (44.4% female, 54.6% male) worms in adult life history stage were noted. From 430 (37.1% female, 62.9% male) adult worms that were chosen for morphological studies 179 (44.4% female, 55.6% male) worms belonged to the German population, 142 (47.4% female, 52.6% male) to Polish and 111 (43.5% female, 56.5% male) to the Taiwanese population.

3.3.1 Differences in the same eel species

3.3.1.1 Body

3.3.1.1.1 Descriptive statistics

The mean, standard error (SE), standard deviation (SD) and minimum and maximum values of the body length, width and length/width ratios of female and male *A. crassus* in the European and Japanese eel are presented in tables 18 and 19, respectively. Generally, the values of the length and width of adults were extremely heterogenic (Figures 22 - 23): the smallest female worm recovered in the European eel was 2 mm in length and 0.2 in the width (German parasite population at 50 dpi) and the biggest was 61 mm in the length (Taiwanese parasite population at 150 dpi) and 4.9 in the width (Polish parasite population at 150 dpi). The smallest male was 3 mm in the length and 0.2 mm in the width and was the same for all 3 parasite populations at 50 dpi. The length of the biggest male amounted 40 mm (Polish parasite population at 100 dpi) and the width was 2.5 mm (German parasite population at 150 dpi).

Table 18: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the body (mm) of females and males of *Anguillicola crassus* in *Anguilla anguilla* . Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	93	16.6	0.7	7.0	2.0	34.0
			Width	92	1.2	0.1	0.6	0.2	2.3
			Length/ width ratio	92	14.3	0.3	3.3	7.8	25.0
		Polish	Length	67	17.2	0.9	7.2	3.0	32.0
			Width	67	1.4	0.1	0.7	0.3	3.1
			Length/ width ratio	67	12.8	0.3	2.6	7.9	20.2
		Taiwanese	Length	30	14.9	1.5	8.4	4.0	36.0
			Width	30	1.1	0.1	0.7	0.3	2.5
			Length/ width ratio	30	15.0	0.7	3.7	6.0	25.0
	Male	German	Length	70	8.7	0.3	2.6	3.0	14.0
			Width	69	0.5	0.0	0.2	0.2	1.0
			Length/ width ratio	69	16.8	0.4	3.3	8.8	29.0
		Polish	Length	85	9.9	0.5	4.2	3.0	20.0
			Width	84	0.7	0.0	0.3	0.2	1.3

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Length/ width ratio	84	15.1	0.5	4.7	7.5	45.0
		Taiwanese	Length	60	8.5	0.4	3.2	3.0	16.0
			Width	60	0.5	0.0	0.2	0.2	1.0
			Length/ width ratio	60	18.1	0.5	3.8	9.2	32.2
100	Female	German	Length	66	28.7	1.2	9.6	8.0	46.0
			Width	67	2.4	0.1	1.0	0.4	4.0
			Length/ width ratio	66	12.5	0.3	2.5	7.9	22.6
		Polish	Length	31	38.2	1.9	10.7	10.0	56.0
			Width	33	2.9	0.2	1.2	0.3	4.6
			Length/ width ratio	31	13.2	0.7	3.9	7.1	28.1
		Taiwanese	Length	35	29.1	1.1	6.8	12.0	40.0
			Width	34	2.5	0.1	0.7	0.9	3.7
			Length/ width ratio	34	12.6	0.6	3.6	7.5	26.2
	Male	German	Length	85	17.5	0.5	4.5	5.0	27.0
			Width	80	1.1	0.0	0.3	0.6	1.7
			Length/ width ratio	80	16.8	0.4	3.4	11.1	29.0
		Polish	Length	54	20.5	1.1	8.2	4.0	40.0
			Width	54	1.2	0.1	0.5	0.3	2.2
			Length/ width ratio	54	17.7	0.6	4.3	11.0	32.8
		Taiwanese	Length	75	15.3	0.7	5.7	5.0	28.0
			Width	75	1.0	0.0	0.4	0.3	1.7
			Length/ width ratio	75	16.0	0.5	4.0	8.5	33.0
150	Female	German	Length	31	37.0	1.5	8.5	18.0	54.0
			Width	32	3.3	0.1	0.7	1.9	4.6
			Length/ width ratio	31	11.3	0.4	2.0	7.2	15.9
		Polish	Length	58	31.6	1.4	10.9	7.0	54.0
			Width	57	2.8	0.2	1.2	0.4	4.9
			Length/ width ratio	57	12.1	0.5	3.6	6.8	28.6
		Taiwanese	Length	37	33.8	2.1	12.7	4.0	61.0
			Width	35	3.0	0.2	0.9	0.9	4.6
			Length/ width ratio	35	12.4	0.6	3.4	6.4	20.9
	Male	German	Length	66	19.5	0.9	7.5	5.0	35.0
			Width	66	1.3	0.1	0.5	0.2	2.5
			Length/ width ratio	66	15.7	0.4	3.4	9.7	27.7
		Polish	Length	83	20.3	0.7	6.4	5.0	36.0

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Width	83	1.4	0.0	0.4	0.4	2.3
			Length/ width ratio	82	15.6	0.3	2.9	6.3	25.7
		Taiwanese	Length	86	19.7	0.8	7.1	5.0	33.0
			Width	87	1.3	0.1	0.5	0.2	2.3
			Length/ width ratio	86	16.1	0.4	3.5	10.0	25.8

In the Japanese eel the smallest female specimen was 4 mm in the length and 0.2 mm in the width (German population at 50 dpi) and the dimensions of the biggest female were 46 mm in the length and 3.1 mm in the width (Polish population at 100 dpi). The smallest male was 2 mm in the length and 0.2 mm in the width (Taiwanese population at 50 dpi). The largest male achieved the length of 23 mm and the width of 1.5 mm (Polish population at 100 dpi).

Table 19: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the body (mm) of females and males of *Anguillicola crassus* in *Anguilla japonica*. Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	39	17.0	1.3	8.2	4.0	31.0
			Width	40	1.2	0.1	0.7	0.2	2.9
			Length/ width ratio	39	16.0	0.6	3.6	9.7	24.3
		Polish	Length	20	8.7	0.7	3.0	5.0	16.0
			Width	21	0.6	0.1	0.3	0.3	1.4
			Length/ width ratio	20	17.0	0.9	4.0	9.0	23.0
		Taiwanese	Length	28	9.3	0.7	3.6	4.0	15.0
			Width	28	0.7	0.1	0.3	0.2	1.2
			Length/ width ratio	28	14.4	0.6	3.1	9.9	20.5
	Male	German	Length	55	8.2	0.3	2.0	4.0	13.0
			Width	56	0.5	0.0	0.1	0.2	0.8
			Length/ width ratio	54	18.4	0.5	3.5	11.4	27.5
		Polish	Length	21	6.2	0.4	1.9	3.0	10.0
			Width	21	0.3	0.0	0.1	0.2	0.5
			Length/ width ratio	21	19.2	0.8	3.8	9.7	26.8
		Taiwanese	Length	28	5.9	0.4	1.9	2.0	9.0
			Width	27	0.4	0.0	0.1	0.2	0.6
			Length/ width ratio	27	15.8	0.6	2.9	9.2	22.5
100	Female	German	Length	7	8.7	0.5	1.4	6.0	10.0
			Width	6	0.5	0.0	0.1	0.3	0.6
			Length/ width ratio	6	18.6	0.9	2.1	14.6	20.0
		Polish	Length	23	22.5	2.3	10.8	5.0	46.0

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Width	21	1.5	0.2	0.8	0.3	3.1
			Length/ width ratio	21	15.1	0.6	2.7	10.5	20.5
		Taiwanese	Length	15	17.3	2.2	8.5	6.0	32.0
			Width	13	1.0	0.1	0.5	0.3	1.7
			Length/ width ratio	13	16.3	0.7	2.5	12.7	20.2
	Male	German	Length	9	8.5	0.7	2.2	6.0	12.0
			Width	9	0.5	0.1	0.2	0.3	0.7
			Length/ width ratio	9	19.3	1.0	3.1	14.6	23.9
		Polish	Length	22	13.4	1.1	5.3	4.0	23.0
			Width	22	0.9	0.1	0.4	0.2	1.5
			Length/ width ratio	22	15.2	0.6	2.8	10.6	22.3
		Taiwanese	Length	24	9.1	1.2	5.8	4.0	22.0
			Width	22	0.5	0.1	0.3	0.3	1.2
			Length/ width ratio	22	16.3	0.6	2.8	10.0	20.8
150	Female	German	Length	8	16.9	2.7	7.7	10.0	34.0
			Width	9	1.3	0.3	0.9	0.5	2.8
			Length/ width ratio	8	16.0	1.3	3.6	11.8	21.1
		Polish	Length	12	27.4	2.4	8.4	7.0	38.0
			Width	13	1.9	0.2	0.8	0.4	2.8
			Length/ width ratio	12	14.2	0.6	2.0	12.0	19.3
		Taiwanese	Length	4	16.3	5.5	11.1	6.0	28.0
			Width	4	1.3	0.5	1.1	0.3	2.5
			Length/ width ratio	4	15.4	2.0	4.1	11.5	20.0
	Male	German	Length	9	8.0	0.5	1.6	6.0	11.0
			Width	12	0.5	0.1	0.2	0.3	0.9
			Length/ width ratio	9	19.6	1.4	4.2	14.3	27.8
		Polish	Length	12	11.0	1.6	5.6	5.0	20.0
			Width	13	0.7	0.1	0.4	0.3	1.5
			Length/ width ratio	12	20.4	1.6	5.4	12.0	29.5
		Taiwanese	Length	7	7.2	1.1	2.9	3.0	11.0
			Width	8	0.6	0.1	0.4	0.3	1.2
			Length/ width ratio	7	15.8	1.7	4.5	10.8	20.0

3.3.1.1.2 Mixed-effects linear models

In both eel species the parasite populations did not differ from one another with respect to length or width of the body (Box 25 - 28; Figures 22 - 23) indicating a lack of genetic divergence for this trait. The only difference found was a smaller body length/width ratio for the Taiwanese population infecting the Japanese eel, indicating a compacter body of these worms compared to both European populations (Box 29, 30). However, as all parasite

populations in the European eel were the same in terms of body shape, evolution of this trait cannot be assumed.

Box 25: Minimal adequate mixed-effects linear model 1: Body length; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	16.491513	0.982622	16.783	**0.0000**
Japanese eel	-5.306911	1.138444	-4.662	**0.0000**
Dpi	0.163275	0.011285	14.469	**0.0000**
Number of adults recovered alive	0.340540	0.094436	3.606	**0.0004**
Number of L3 recovered alive	-0.178437	0.060299	-2.959	**0.0034**
Male	-8.107014	0.573146	-14.145	**0.0000**
Japanese eel*Dpi	-0.081702	0.018806	-4.344	**0.0000**
Dpi*Male	-0.058750	0.008704	-6.750	**0.0000**
Japanese eel*Male	2.945838	0.828518	3.556	**0.0004**

Box 26: Minimal adequate mixed-effects linear model 2: Body width; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	1.314667	0.078705	16.704	**0.0000**
Japanese eel	-0.608424	0.091783	-6.629	**0.0000**
Dpi	0.016057	0.000910	17.649	**0.0000**
Number of adults recovered alive	0.020771	0.007476	2.778	**0.0059**
Number of L3 recovered alive	-0.012964	0.004764	-2.721	**0.0069**
Male	-0.816279	0.049885	-16.363	**0.0000**
Japanese eel*Dpi	-0.008335	0.001509	-5.523	**0.0000**
Dpi*Male	-0.008211	0.000758	-10.839	**0.0000**
Japanese eel*Male	0.531043	0.072220	7.353	**0.0000**

Box 27: Minimal adequate mixed-effects linear model 3: Body ratio; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	13.641913	0.368998	36.970	**0.0000**
Japanese eel	2.299808	0.656454	3.503	**0.0005**
German parasite population	0.351104	0.384348	0.914	0.3618
Taiwanese parasite population	0.766878	0.415363	1.846	0.0660
Dpi	-0.017952	0.004705	-3.816	**0.0002**
Male	2.515979	0.294228	8.551	**0.0000**
Japanese eel*German parasite population	0.565012	0.721843	0.783	0.4345

Explanatory variables and interactions	Estimate	SD	t-value	p-value
Japanese eel*Taiwanese parasite population	-2.260585	0.759629	-2.976	**0.0032**
Japanese eel*Dpi	0.017957	0.007626	2.355	**0.0193**
Dpi*Male	0.011858	0.004458	2.660	**0.0079**
Japanese eel*Male	-1.012030	0.422709	-2.394	**0.0168**

Box 28: Minimal adequate mixed-effects linear model 10: Bodylength; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	16.491513	0.982622	16.783	**0.0000**
Japanese eel	-5.306911	1.138444	-4.662	**0.0000**
Dpi	0.163275	0.011285	14.469	**0.0000**
Number of adults recovered alive	0.340540	0.094436	3.606	**0.0004**
Number of L3 recovered alive	-0.178437	0.060299	-2.959	**0.0034**
Male	-8.107014	0.573146	-14.145	**0.0000**
Japanese eel*Dpi	-0.081702	0.018806	-4.344	**0.0000**
Dpi*Male	-0.058750	0.008704	-6.750	**0.0000**
Japanese eel*Male	2.945838	0.828518	3.556	**0.0004**

Box 29: Minimal adequate mixed-effects linear model 11: Bodywidth; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	1.314667	0.078705	16.704	**0.0000**
Japanese eel	-0.608424	0.091783	-6.629	**0.0000**
Dpi	0.016057	0.000910	17.649	**0.0000**
Number of adults recovered alive	0.020771	0.007476	2.778	**0.0059**
Number of L3 recovered alive	-0.012964	0.004764	-2.721	**0.0069**
Male	-0.816279	0.049885	-16.363	**0.0000**
Japanese eel*Dpi	-0.008335	0.001509	-5.523	**0.0000**
Dpi*Male	-0.008211	0.000758	-10.839	**0.0000**
Japanese eel*Male	0.531043	0.072220	7.353	**0.0000**

Box 30: Minimal adequate mixed-effects linear model 12: Bodyratio; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	13.993017	0.344908	40.570	**0.0000**
Japanese eel	2.864821	0.597873	4.792	**0.0000**
Polish parasite population	-0.351104	0.384348	-0.914	0.3618

Explanatory variables and interactions	Estimate	SD	t-value	p-value
Taiwanese parasite population	0.415774	0.407112	1.021	0.3081
Dpi	-0.017952	0.004705	-3.816	**0.0002**
Male	2.515979	0.294228	8.551	**0.0000**
Japanese eel*Polish parasite population	-0.565012	0.721843	-0.783	0.4345
Japanese eel*Taiwanese parasite population	-2.825598	0.738580	-3.826	**0.0002**
Japanese eel*Dpi	0.017957	0.007626	2.355	**0.0193**
Dpi*Male	0.011858	0.004458	2.660	**0.0079**
Japanese eel*Male	-1.012030	0.422709	-2.394	**0.0168**

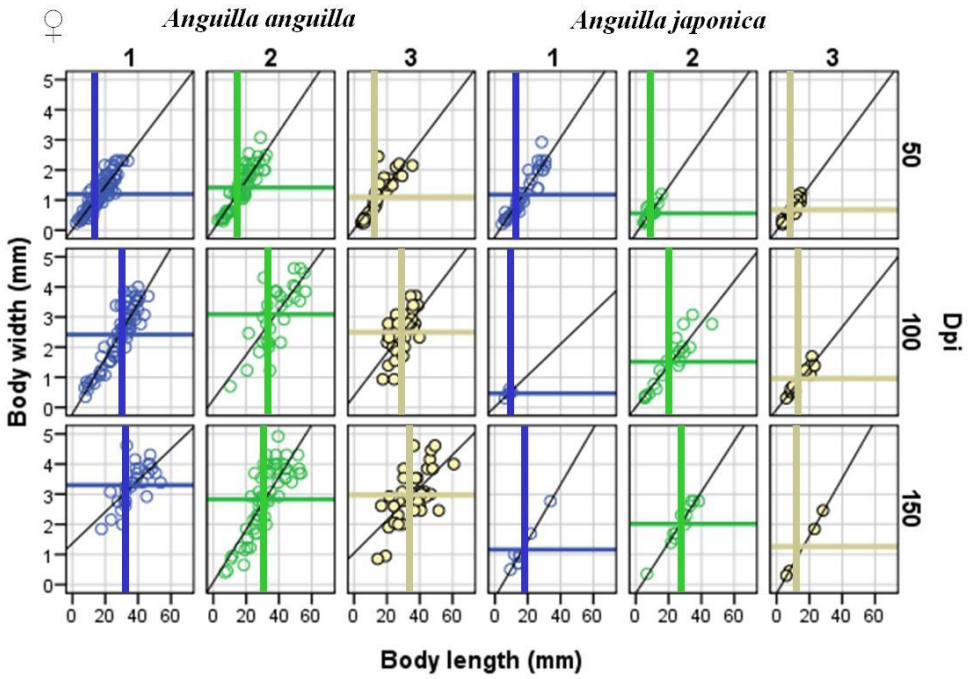

Figure 20: Body length and body width of female adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

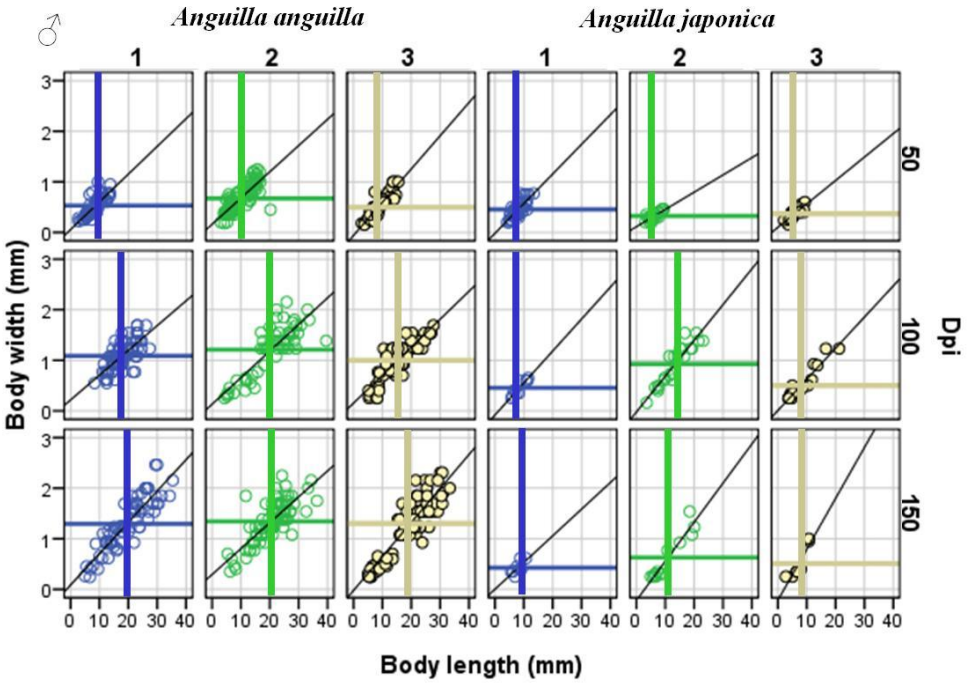

Figure 21: Body length and body width of male adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

3.3.1.2 Oesophagus

3.3.1.2.1 Descriptive statistics

As with the body, the oesophageal dimensions showed a high degree of variability (Figure 24 - 25).The mean, standard error (SE), standard deviation (SD) and minimum and maximum values of length, width and length/width ratios of the body of female and male *A. crassus* in the European and Japanese eel are presented in tables 20 and 21, respectively. In the European eel the smallest oesophagus was 540 µm in the length and 110 µm in the width and belonged to the Taiwanese population at 50 dpi. The largest female oesophagus had the length of 1,170 µm (Polish population at 150 dpi) and the width of 510 µm (Taiwanese population at 150 dpi). In case of males, 460 µm in the length and 110 µm in the width were dimensions of the smallest oesophagus from the Taiwanese population at 50 dpi. The largest male oesophagus was noted for Polish population at 150 dpi and was 1,000 µm in the length and 410 µm in the width.

Table 20: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the oesophagus (µm) of females and males of *Anguillicola crassus* in *Anguilla anguilla* . Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	89	710.6	6.8	64.3	540.0	880.0
			Width	91	214.4	3.0	28.3	140.0	270.0
			Length/ width ratio	87	3.4	0.04	0.3	2.8	4.5
		Polish	Length	58	769.5	9.1	69.1	590.0	950.0
			Width	58	252.2	5.0	38.3	170.0	320.0
			Length/ width ratio	57	3.1	0.05	0.4	2.0	3.9
		Taiwanese	Length	28	700.7	15.8	83.8	540.0	870.0
			Width	27	202.6	6.7	34.9	110.0	280.0
			Length/ width ratio	27	3.5	0.1	0.5	2.6	5.2
	Male	German	Length	68	610.4	6.4	53.0	470.0	730.0
			Width	64	186.9	3.2	25.5	140.0	240.0
			Length/ width ratio	63	3.3	0.05	0.4	2.5	4.7
		Polish	Length	73	655.9	7.5	64.0	510.0	800.0
			Width	71	207.9	3.5	29.6	150.0	270.0
			Length/ width ratio	71	3.2	0.04	0.3	2.3	4.0
		Taiwanese	Length	59	591.2	7.8	59.8	460.0	720.0
			Width	59	181.0	4.3	33.3	110.0	240.0
			Length/ width ratio	58	3.3	0.1	0.4	2.7	4.2
100	Female	German	Length	62	858.4	11.7	92.5	650.0	1,080.0
			Width	62	283.5	6.2	49.2	160.0	400.0
			Length/ width ratio	61	3.1	0.05	0.4	2.6	4.5
		Polish	Length	30	914.0	18.8	103.1	690.0	1,110.0
			Width	30	313.0	8.9	49.0	200.0	400.0
			Length/ width ratio	30	3.0	0.1	0.3	2.5	3.7
		Taiwanese	Length	34	854.1	16.0	93.2	650.0	1,020.0
			Width	33	288.8	7.7	44.4	200.0	380.0
			Length/ width ratio	33	3.0	0.1	0.4	2.2	3.9
	Male	German	Length	82	734.1	7.1	64.0	560.0	880.0
			Width	83	254.2	3.9	35.9	160.0	350.0
			Length/ width ratio	81	2.9	0.03	0.3	2.3	4.0
		Polish	Length	54	781.7	13.5	99.3	590.0	1,000.0
			Width	54	284.8	7.5	54.9	170.0	400.0

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Length/ width ratio	54	2.8	0.05	0.3	2.2	3.6
		Taiwanese	Length	71	731.4	8.4	70.6	540.0	860.0
			Width	70	256.1	5.0	42.1	150.0	340.0
			Length/ width ratio	69	2.9	0.04	0.4	2.3	4.1
150	Female	German	Length	26	946.5	14.4	73.3	790.0	1,110.0
			Width	26	336.5	10.6	54.0	250.0	470.0
			Length/ width ratio	25	2.9	0.1	0.3	2.2	3.6
		Polish	Length	45	967.6	16.8	112.8	700.0	1,170.0
			Width	45	332.4	8.7	58.5	210.0	440.0
			Length/ width ratio	43	2.9	0.1	0.4	2.3	4.6
		Taiwanese	Length	28	900.4	24.0	127.0	600.0	1,110.0
			Width	25	360.8	17.4	87.2	180.0	510.0
			Length/ width ratio	25	2.6	0.1	0.4	2.0	3.3
	Male	German	Length	64	782.0	12.3	98.7	510.0	980.0
			Width	64	269.4	8.8	70.8	110.0	470.0
			Length/ width ratio	64	3.0	0.1	0.6	2.1	5.3
		Polish	Length	75	824.4	8.2	70.8	690.0	1,000.0
			Width	71	313.8	4.8	40.7	230.0	410.0
			Length/ width ratio	70	2.6	0.03	0.2	2.2	3.2
		Taiwanese	Length	80	786.7	9.2	82.4	570.0	930.0
			Width	80	270.9	6.3	56.1	150.0	390.0
			Length/ width ratio	79	3.0	0.05	0.4	2.1	4.8

In the Japanese eel the smallest female oesophagus was 530 µm in the length and 90 µm in the width (German population at 50 dpi) and the biggest achieved dimensions of 1060 µm and 360 µm for the length and width, respectively (Taiwanese population at 150 dpi). For the males, the smallest oesophagus belonged to the Taiwanese population at 50 dpi and its dimensions were 470 µm in the length and 120 µm in the width. 890 µm in the length (Taiwanese population at 150 dpi) and 280 µm in the width were dimensions of the largest oesophagus of males in this eel species (Polish population at 150 dpi).

Table 21: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the oesophagus (µm) of females and males of *Anguillicola crassus* in *Anguilla japonica*. Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	39	709.5	13.5	84.5	530.0	870.0
			Width	40	200.3	7.9	50.0	90.0	310.0
			Length/ width ratio	39	3.8	0.1	0.8	3.0	6.6
		Polish	Length	21	680.5	16.3	74.7	560.0	810.0
			Width	21	188.6	6.9	31.5	110.0	230.0
			Length/ width ratio	21	3.7	0.1	0.4	3.1	5.2
		Taiwanese	Length	25	692.8	12.2	61.1	560.0	800.0
			Width	27	188.9	7.5	39.2	100.0	250.0
			Length/ width ratio	25	3.6	0.1	0.4	3.0	5.0
	Male	German	Length	51	622.8	5.4	38.4	530.0	710.0
			Width	55	173.1	3.0	22.6	120.0	220.0
			Length/ width ratio	50	3.6	0.1	0.4	2.9	4.5
		Polish	Length	20	614.0	12.3	55.1	510.0	700.0
			Width	19	168.4	2.7	11.7	150.0	190.0
			Length/ width ratio	19	3.6	0.1	0.4	3.1	4.5
		Taiwanese	Length	28	579.3	9.5	50.0	470.0	680.0
			Width	26	159.6	3.1	15.9	120.0	190.0
			Length/ width ratio	26	3.6	0.04	0.2	3.2	4.1
100	Female	German	Length	7	701.4	49.2	130.2	570.0	900.0
			Width	7	188.6	27.4	72.4	120.0	290.0
			Length/ width ratio	7	4.0	0.3	0.9	2.9	5.4
		Polish	Length	20	845.0	25.1	112.3	650.0	1,050.0
			Width	21	247.6	9.6	43.9	160.0	320.0
			Length/ width ratio	20	3.5	0.1	0.4	2.5	4.3
		Taiwanese	Length	14	747.9	17.7	66.4	620.0	840.0
			Width	14	218.6	8.4	31.3	180.0	280.0
			Length/ width ratio	14	3.5	0.1	0.3	3.0	3.9
	Male	German	Length	8	666.3	14.1	40.0	610.0	730.0
			Width	8	192.5	4.5	12.8	170.0	210.0
			Length/ width ratio	8	3.5	0.1	0.3	3.1	3.8
		Polish	Length	21	747.1	12.7	58.3	650.0	850.0
			Width	21	218.1	5.9	27.1	160.0	260.0

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Length/ width ratio	21	3.5	0.1	0.3	2.9	4.3
		Taiwanese	Length	24	696.3	20.3	99.6	470.0	850.0
			Width	24	209.6	7.6	37.1	120.0	270.0
			Length/ width ratio	24	3.4	0.1	0.3	2.8	4.3
150	Female	German	Length	9	781.1	19.3	58.0	720.0	860.0
			Width	9	234.4	13.9	41.6	180.0	300.0
			Length/ width ratio	9	3.4	0.1	0.4	2.8	4.0
		Polish	Length	11	890.0	19.8	65.7	820.0	1020.0
			Width	13	245.4	12.8	46.3	150.0	310.0
			Length/ width ratio	11	3.5	0.1	0.3	3.0	4.1
		Taiwanese	Length	4	832.5	128.5	257.1	600.0	1,060.0
			Width	4	227.5	52.3	104.7	110.0	350.0
			Length/ width ratio	4	4.0	0.5	1.1	3.0	5.5
	Male	German	Length	11	643.6	10.5	34.7	600.0	700.0
			Width	12	200.0	7.8	27.0	170.0	250.0
			Length/ width ratio	11	3.3	0.1	0.3	2.9	3.8
		Polish	Length	13	704.6	23.2	83.6	610.0	880.0
			Width	13	216.2	8.6	31.0	170.0	280.0
			Length/ width ratio	13	3.3	0.1	0.2	2.8	3.6
		Taiwanese	Length	8	707.5	37.8	106.9	550.0	890.0
			Width	6	215.0	14.3	35.1	170.0	260.0
			Length/ width ratio	6	3.6	0.3	0.7	2.6	4.7

3.3.1.2.2 Mixed-effects linear models

In both eel species the Polish population had a longer and wider oesophagus and a lower oesophagus length/width ratio than the German and Taiwanese populations (Boxes 31, 32, 34, 35; Figures 24, 25). There were no differences in length, width and length/width ratio of the oesophagus between the German and Taiwanese populations (Boxes 34 - 36). The larger dimensions and more compact shape of the oesophagus of the Polish parasite population appear to be genetically fixed traits.

Box 31: Minimal adequate mixed-effects linear model 4: Oesophagus length; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	746.8097	12.689587	58.852	**0.0000**
Japanese eel	-20.6429	12.403781	-1.664	0.0973
German parasite population	-40.2470	9.484653	-4.243	**0.0000**
Taiwanese parasite population	-35.8230	11.510926	-3.112	**0.0021**
Dpi	2.0248	0.136997	14.780	**0.0000**
Number of adults recovered alive	3.6390	1.111863	3.273	**0.0012**
Number of L3 recovered alive	-2.1433	0.849951	-2.522	**0.0123**
Male	-100.8857	6.227117	-16.201	**0.0000**
Japanese eel*Dpi	-0.8783	0.219523	-4.001	**0.0001**
Dpi*Male	-0.3637	0.108472	-3.353	**0.0008**

Box 32: Minimal adequate mixed-effects linear model 5: Oesophagus width; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	235.50228	7.177632	32.811	**0.0000**
Japanese eel	-26.70000	7.090922	-3.765	**0.0002**
German parasite population	-22.69677	5.500497	-4.126	**0.0000**
Taiwanese parasite population	-27.34239	5.878524	-4.651	**0.0000**
Dpi	0.96332	0.078655	12.248	**0.0000**
Number of adults recovered alive	1.30643	0.632441	2.066	**0.0399**
Male	-25.48513	3.606559	-7.066	**0.0000**
Japanese eel*Dpi	-0.46864	0.128165	-3.657	**0.0003**
Dpi*Male	-0.16827	0.062968	-2.672	**0.0076**

Box 33: Minimal adequate mixed-effects linear model 6: Oesophagus ratio; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	3.164849	0.034298	92.275	**0.0000**
Japanese eel	0.354854	0.043646	8.130	**0.0000**
German parasite population	0.152373	0.033900	4.495	**0.0000**
Taiwanese parasite population	0.121945	0.036481	3.343	**0.0010**
Dpi	-0.004029	0.000415	-9.701	**0.0000**
Male	-0.048802	0.022015	-2.217	**0.0268**
Japanese eel*Dpi	0.002353	0.000802	2.933	**0.0037**

Box 34: Minimal adequate mixed-effects linear model 13: Oesophagus length; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	706.5627	12.045157	58.659	**0.0000**
Japanese eel	-20.6429	12.403781	-1.664	0.0973
Polish parasite population	40.2470	9.484653	4.243	**0.0000**
Taiwanese parasite population	4.4241	11.557874	0.383	0.7022
Dpi	2.0248	0.136997	14.780	**0.0000**
Number of adults recovered alive	3.6390	1.111863	3.273	**0.0012**
Number of L3 recovered alive	-2.1433	0.849951	-2.522	**0.0123**
Male	-100.8857	6.227117	-16.201	**0.0000**
Japanese eel*Dpi	-0.8783	0.219523	-4.001	**0.0001**
Dpi*Male	-0.3637	0.108472	-3.353	**0.0008**

Box 35: Minimal adequate mixed-effects linear model 14: Oesophagus width; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	212.80550	6.870383	30.974	**0.0000**
Japanese eel	-26.70000	7.090922	-3.765	**0.0002**
Polish parasite population	22.69677	5.500497	4.126	**0.0000**
Taiwanese parasite population	-4.64561	5.702139	-0.815	0.4160
Dpi	0.96332	0.078655	12.248	**0.0000**
Number of adults recovered alive	1.30643	0.632441	2.066	**0.0399**
Male	-25.48513	3.606559	-7.066	**0.0000**
Japanese eel*Dpi	-0.46864	0.128165	-3.657	**0.0003**
Dpi*Male	-0.16827	0.062968	-2.672	**0.0076**

Box 36: Minimal adequate mixed-effects linear model 15: Oesophagus ratio; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	3.317222	0.032135	103.229	**0.0000**
Japanese eel	0.354854	0.043646	8.130	**0.0000**
Polish parasite population	-0.152373	0.033900	-4.495	**0.0000**
Taiwanese parasite population	-0.030428	0.035232	-0.864	0.3886
Dpi	-0.004029	0.000415	-9.701	**0.0000**
Male	-0.048802	0.022015	-2.217	**0.0268**
Japanese eel*Dpi	0.002353	0.000802	2.933	**0.0037**

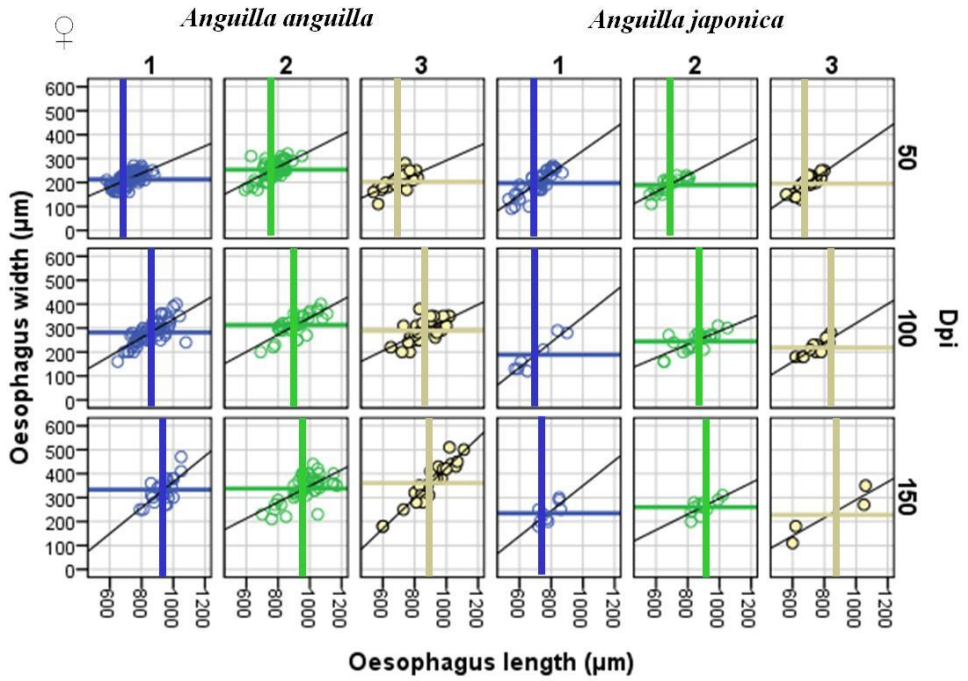

Figure 22: Oesophagus length and oesophagus width of female adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

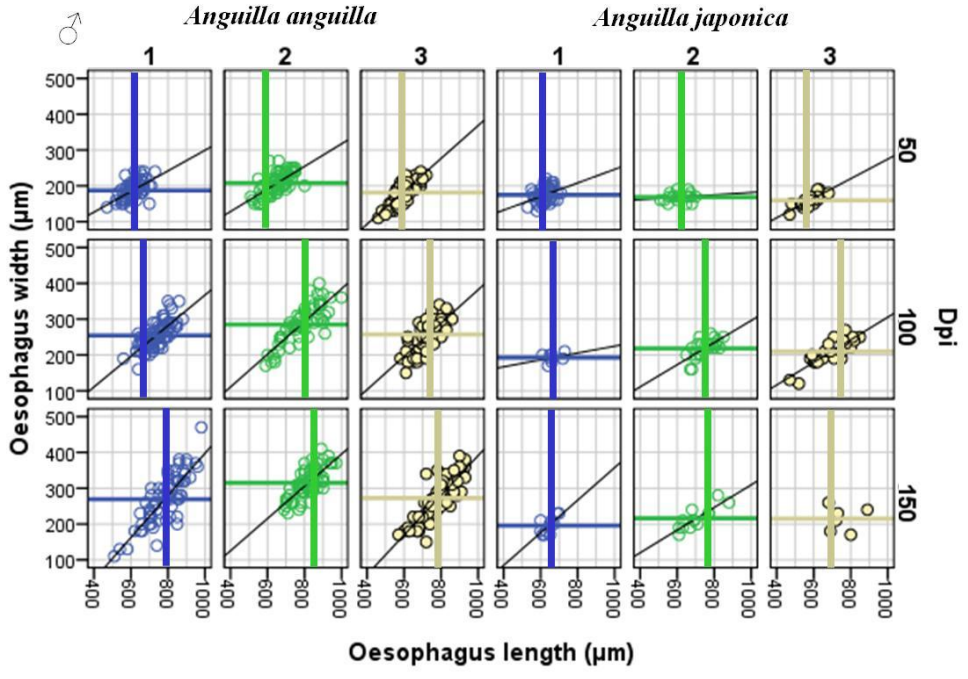

Figure 23: Oesophagus length and oesophagus width of male adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

3.3.1.3 Buccal capsule

3.3.1.3.1 Descriptive statistics

The measurements of the buccal capsule displayed higher degree of homogeneity than those of the body and oesophagus (Figure 26 - 27). The mean, standard error (SE), standard deviation (SD) and minimum and maximum values of length, width and length/width ratios of the body of the females and males *A. crassus* in both eel species are presented in tables 22 and 23. In the European eel the smallest female buccal capsule was 15 µm in the length and 40 µm in the width and belonged to the German and Taiwanese populations at 50 dpi. The largest female buccal capsule was 27 µm in the length (Polish population at 150 dpi) and 70 µm in the width (Polish population at 50 dpi). For the males, the smallest dimensions were noted for the Taiwanese population at 50 dpi: 14 µm for the length and 37 µm for the width. The largest male buccal capsule was 26 µm in the length (Taiwanese population at 150 dpi) and 65 µm in the width (Polish population at 100 dpi and Taiwanese population at 150 dpi).

Table 22: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the buccal capsule (µm) of females and males of *Anguillicola crassus* in *Anguilla anguilla*. Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	93	19.7	0.19	1.85	15.0	23.0
			Width	93	53.1	0.59	5.66	40.0	68.0
			Length/ width ratio	93	0.4	0.003	0.03	0.3	0.5
		Polish	Length	65	21.0	0.22	1.78	17.0	25.0
			Width	66	56.6	0.73	5.97	42.0	70.0
			Length/ width ratio	64	0.4	0.004	0.03	0.3	0.5
		Taiwanese	Length	28	20.1	0.39	2.09	15.0	25.0
			Width	28	53.9	1.27	6.71	40.0	66.0
			Length/ width ratio	28	0.4	0.01	0.03	0.3	0.4
	Male	German	Length	64	19.4	0.21	1.69	15.0	23.0
			Width	65	50.0	0.69	5.53	39.0	62.0
			Length/ width ratio	64	0.4	0.004	0.03	0.3	0.5
		Polish	Length	81	19.9	0.18	1.60	16.0	24.0
			Width	80	51.9	0.55	4.90	43.0	64.0
			Length/ width ratio	80	0.4	0.004	0.03	0.3	0.5
		Taiwanese	Length	61	18.4	0.25	1.94	14.0	23.0

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
			Width	60	47.6	0.66	5.08	37.0	60.0
			Length/ width ratio	60	0.4	0.003	0.03	0.3	0.4
100	Female	German	Length	63	20.0	0.24	1.90	16.0	24.0
			Width	62	52.0	0.57	4.46	44.0	62.0
			Length/ width ratio	61	0.4	0.004	0.03	0.3	0.5
		Polish	Length	29	21.2	0.43	2.29	16.0	25.0
			Width	30	56.6	1.20	6.57	44.0	69.0
			Length/ width ratio	29	0.4	0.01	0.03	0.3	0.4
		Taiwanese	Length	32	20.5	0.23	1.32	18.0	23.0
			Width	36	54.4	0.86	5.14	46.0	69.0
			Length/ width ratio	32	0.4	0.01	0.03	0.3	0.4
	Male	German	Length	82	19.7	0.23	2.11	15.0	25.0
			Width	82	49.7	0.61	5.49	39.0	61.0
			Length/ width ratio	82	0.4	0.003	0.03	0.3	0.5
		Polish	Length	52	20.4	0.24	1.74	17.0	25.0
			Width	53	52.7	0.72	5.24	42.0	65.0
			Length/ width ratio	52	0.4	0.01	0.04	0.3	0.5
		Taiwanese	Length	73	19.6	0.19	1.63	15.0	23.0
			Width	74	50.9	0.55	4.71	39.0	63.0
			Length/ width ratio	73	0.4	0.003	0.03	0.3	0.5
150	Female	German	Length	30	20.5	0.46	2.54	16.0	25.0
			Width	26	53.4	0.72	3.68	45.0	61.0
			Length/ width ratio	26	0.4	0.01	0.04	0.3	0.5
		Polish	Length	46	22.0	0.30	2.05	17.0	27.0
			Width	44	56.2	0.62	4.14	47.0	66.0
			Length/ width ratio	42	0.4	0.005	0.03	0.3	0.4
		Taiwanese	Length	29	20.0	0.46	2.49	15.0	23.0
			Width	30	53.3	1.23	6.72	41.0	66.0
			Length/ width ratio	29	0.4	0.01	0.04	0.3	0.5
	Male	German	Length	53	19.7	0.25	1.82	17.0	26.0
			Width	57	48.7	0.58	4.39	39.0	60.0
			Length/ width ratio	52	0.4	0.004	0.03	0.4	0.5
		Polish	Length	74	20.0	0.23	1.95	15.0	25.0
			Width	74	50.5	0.52	4.48	39.0	59.0
			Length/ width ratio	73	0.4	0.003	0.03	0.3	0.5

Dpi	Worm sex	Parasite population	Measurement	N	Mean	SE	SD	Minimum	Maximum
		Taiwanese	Length	81	19.6	0.25	2.28	15.0	25.0
			Width	80	50.6	0.65	5.84	39.0	65.0
			Length/ width ratio	80	0.4	0.004	0.04	0.3	0.5

In the Japanese eel the smallest female buccal capsule was 15 µm in the length (German and Taiwanese populations at 50 dpi) and 40 µm in the width (German population at 50 dpi). 27 µm and 72 µm were dimensions of the largest female buccal capsule noted for the Polish population at 100 dpi. For the males the smallest buccal capsule was 12 µm and 38 µm in the length and width, respectively, and was noted for the Taiwanese population at 50 dpi. The largest male buccal capsule was 24 µm in the length and 62 µm in the width and belonged to the Taiwanese population at 150 dpi.

Table 23: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the buccal capsule (µm) of females and males of *Anguillicola crassus* from Germany, Poland and Taiwan in *Anguilla japonica*. Dpi - days post infection.

Dpi	Worm sex	Parasite population	Measurements	N	Mean	SE	SD	Minimum	Maximum
50	Female	German	Length	40	19.4	0.36	2.30	15.0	24.0
			Width	40	50.1	0.88	5.54	40.0	63.0
			Length/ width ratio	40	0.4	0.008	0.02	0.3	0.4
		Polish	Length	20	20.6	0.47	2.11	17.0	25.0
			Width	18	55.4	0.75	3.19	50.0	62.0
			Length/ width ratio	18	0.4	0.01	0.03	0.3	0.4
		Taiwanese	Length	27	19.2	0.36	1.85	15.0	23.0
			Width	27	51.4	1.09	5.64	41.0	62.0
			Length/ width ratio	27	0.4	0.01	0.03	0.3	0.4
	Male	German	Length	58	18.9	0.22	1.71	15.0	23.0
			Width	56	48.2	0.50	3.72	41.0	57.0
			Length/ width ratio	56	0.4	0.001	0.03	0.3	0.5
		Polish	Length	19	19.1	0.40	1.73	15.0	21.0
			Width	20	51.8	1.57	7.02	39.0	66.0
			Length/ width ratio	19	0.4	0.01	0.03	0.3	0.5
		Taiwanese	Length	28	18.6	0.51	2.67	12.0	23.0
			Width	28	48.3	1.00	5.30	38.0	60.0

Dpi	Worm sex	Parasite population	Measurements	N	Mean	SE	SD	Minimum	Maximum
			Length/ width ratio	28	0.4	0.01	0.04	0.3	0.5
100	Female	German	Length	8	20.6	0.42	1.19	19.0	22.0
			Width	8	54.6	1.57	4.44	48.0	61.0
			Length/ width ratio	8	0.4	0.01	0.02	0.3	0.4
		Polish	Length	20	22.6	0.48	2.14	20.0	27.0
			Width	22	58.7	1.34	6.31	48.0	72.0
			Length/ width ratio	20	0.4	0.008	0.02	0.3	0.4
		Taiwanese	Length	13	22.6	0.54	1.94	19.0	26.0
			Width	12	55.1	1.16	4.01	48.0	62.0
			Length/ width ratio	12	0.4	0.01	0.03	0.4	0.5
	Male	German	Length	7	20.6	0.20	0.54	20.0	21.0
			Width	8	52.3	1.25	3.54	47.0	58.0
			Length/ width ratio	7	0.4	0.01	0.03	0.4	0.4
		Polish	Length	22	20.8	0.41	1.94	17.0	24.0
			Width	20	53.6	0.90	4.04	45.0	58.0
			Length/ width ratio	20	0.4	0.006	0.02	0.4	0.4
		Taiwanese	Length	22	20.5	0.31	1.44	18.0	23.0
			Width	24	53.1	1.08	5.31	43.0	66.0
			Length/ width ratio	22	0.4	0.01	0.04	0.3	0.5
150	Female	German	Length	9	22.0	0.75	2.24	18.0	25.0
			Width	9	57.7	1.86	5.59	50.0	66.0
			Length/ width ratio	9	0.4	0.01	0.03	0.3	0.4
		Polish	Length	13	22.0	0.44	1.58	20.0	25.0
			Width	13	56.5	1.28	4.63	50.0	65.0
			Length/ width ratio	13	0.4	0.01	0.03	0.3	0.5
		Taiwanese	Length	4	21.5	0.65	1.29	20.0	23.0
			Width	4	57.3	1.11	2.22	55.0	60.0
			Length/ width ratio	4	0.4	0.01	0.02	0.4	0.4
	Male	German	Length	11	20.5	0.37	1.21	18.0	22.0
			Width	12	52.3	1.23	4.25	48.0	60.0
			Length/ width ratio	11	0.4	0.01	0.03	0.4	0.4
		Polish	Length	13	21.1	0.51	1.85	18.0	25.0
			Width	13	54.8	1.03	3.72	50.0	61.0
			Length/ width ratio	13	0.4	0.01	0.02	0.4	0.4
		Taiwanese	Length	8	20.4	0.78	2.20	17.0	24.0

Dpi	Worm sex	Parasite population	Measurements	N	Mean	SE	SD	Minimum	Maximum
			Width	8	52.0	2.35	6.66	42.0	62.0
			Length/ width ratio	8	0.4	0.01	0.04	0.4	0.5

3.3.1.3.2 Mixed-effects linear models

In both eel species the Polish population had a longer and wider buccal capsule than the German and Taiwanese populations (Boxes 37, 38, 40, 41; Figures 26 - 27), and the Taiwanese worms had longer buccal capsules than their German counterparts, but no differences in the width between these two populations could be found (Boxes 40, 41; Figures 26 - 27). However, the high significance level (0.08) of the positive influence of the Taiwanese population on the width of the buccal capsule suggests a trend directed to larger buccal capsules in the Taiwanese population compared with the German one. As the differences in dimensions of the buccal capsule appeared when infecting the same eel species, a morphological divergence between the Polish and the German populations and between the Polish and the Taiwanese populations in respect to this trait is indicated.

The buccal capsule length/width ratios of the Polish and Taiwanese populations were lower than for the German population but no differences between the Polish and Taiwanese populations could be found (Boxes 39, 42). The Polish and Taiwanese populations have not diverged in respect to the shape of buccal capsule, while the German population appears to have evolved a more compact buccal capsule in comparison to its Polish and Taiwanese conspecific.

Box 37: Minimal adequate mixed-effects linear model 7: Buccal capsule length; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	21.464608	0.229904	93.363	**0.0000**
Japanese eel	-0.397026	0.227728	-1.743	0.0824
German parasite population	-1.013310	0.174266	-5.815	**0.0000**
Taiwanese parasite population	-0.485218	0.214307	-2.264	**0.0244**
Dpi	0.005815	0.002190	2.655	**0.0084**
Number of adults recovered alive	-0.044958	0.020109	-2.236	**0.0262**
Number of L3 recovered alive	-0.054007	0.015478	-3.489	**0.0006**
Male	-0.771953	0.107134	-7.206	**0.0000**
Japanese eel*Dpi	0.010065	0.004207	2.393	**0.0174**

Box 38: Minimal adequate mixed-effects linear model 8: Buccal capsule width; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	57.58608	0.618577	93.095	**0.0000**
Japanese eel	-1.18084	0.644167	-1.833	0.0679
German parasite population	-4.03245	0.702212	-5.743	**0.0000**
Taiwanese parasite population	-2.65929	0.814654	-3.264	**0.0012**
Dpi	-0.01357	0.009125	-1.487	0.1383
Number of L3 recovered alive	-0.14712	0.043289	-3.399	**0.0008**
Male	-3.81353	0.288494	-13.219	**0.0000**
Japanese eel*Dpi	0.03459	0.011475	3.014	**0.0028**
German parasite population*Dpi	0.01721	0.011988	1.435	0.1524
Taiwanese parasite population*Dpi	0.03320	0.013075	2.539	**0.0117**

Box 39: Minimal adequate mixed-effects linear model 9: Buccal capsule ratio; reference group: Polish parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	0.370842	0.002395	154.809	**0.0000**
Japanese eel	0.009065	0.003123	2.902	**0.0040**
German parasite population	0.004939	0.002317	2.131	**0.0340**
Taiwanese parasite population	-0.000080	0.002463	-0.033	0.9741
Dpi	0.000078	0.000024	3.214	**0.0015**
Male	0.015202	0.002018	7.534	**0.0000**
Japanese eel*Male	-0.012043	0.003956	-3.044	**0.0024**

Box 40: Minimal adequate mixed-effects linear model 16: Buccal capsule length; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	20.451298	0.217661	93.959	**0.0000**
Japanese eel	-0.397026	0.227728	-1.743	0.0824
Polish parasite population	1.013310	0.174266	5.815	**0.0000**
Taiwanese parasite population	0.528092	0.214901	2.457	**0.0146**
Dpi	0.005815	0.002190	2.655	**0.0084**
Number of adults recovered alive	-0.044958	0.020109	-2.236	**0.0262**
Number of L3 recovered alive	-0.054007	0.015478	-3.489	**0.0006**
Male	-0.771953	0.107134	-7.206	**0.0000**
Japanese eel*Dpi	0.010065	0.004207	2.393	**0.0174**

Box 41: Minimal adequate mixed-effects linear model 17: Buccal capsule width; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	53.55363	0.572313	93.574	**0.0000**
Japanese eel	-1.18084	0.644167	-1.833	0.0679
Polish parasite population	4.03245	0.702212	5.743	**0.0000**
Taiwanese parasite population	1.37316	0.781350	1.757	0.0800
Dpi	0.00364	0.009154	0.398	0.6912
Number of L3 recovered alive	-0.14712	0.043289	-3.399	**0.0008**
Male	-3.81353	0.288494	-13.219	**0.0000**
Japanese eel*Dpi	0.03459	0.011475	3.014	**0.0028**
Polish parasite population*Dpi	-0.01721	0.011988	-1.435	0.1524
Taiwanese parasite population*Dpi	0.01599	0.012861	1.244	0.2148

Box 42: Minimal adequate mixed-effects linear model 18: Buccal capsule ratio; reference group: German parasite population in the European eel. Significant effects are in bold.

Explanatory variables and interactions	Estimate	SD	t-value	p-value
(Intercept)	0.375781	0.002190	171.579	**0.0000**
Japanese eel	0.009065	0.003123	2.902	**0.0040**
Polish parasite population	-0.004939	0.002317	-2.131	**0.0340**
Taiwanese parasite population	-0.005019	0.002402	-2.089	**0.0377**
Dpi	0.000078	0.000024	3.214	**0.0015**
Male	0.015202	0.002018	7.534	**0.0000**
Japanese eel*Male	-0.012043	0.003956	-3.044	**0.0024**

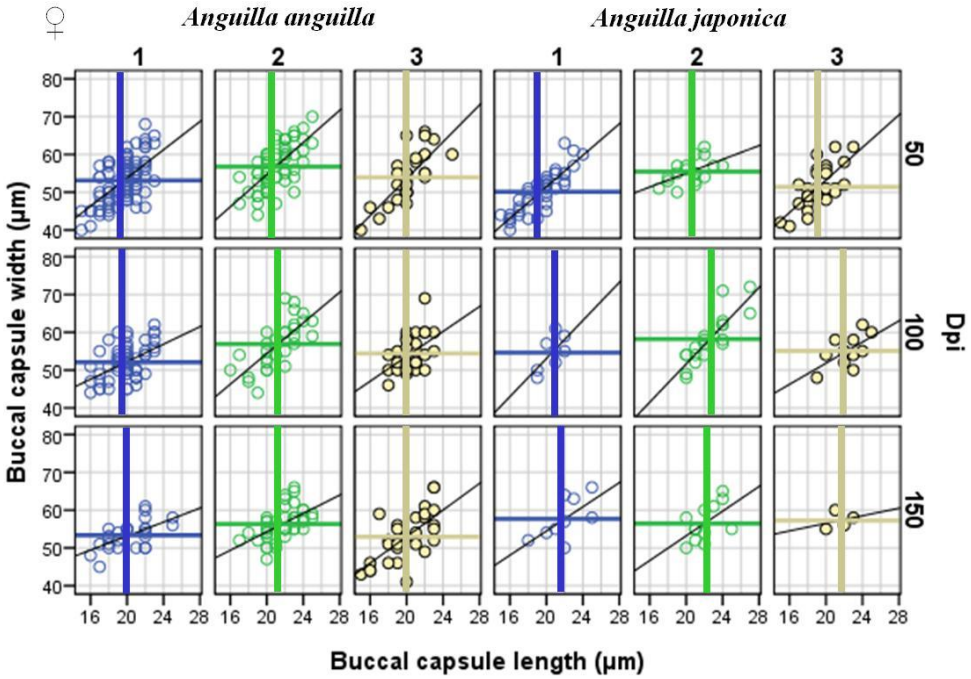

Figure 24: Buccal capsule length and buccal capsule width of female adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

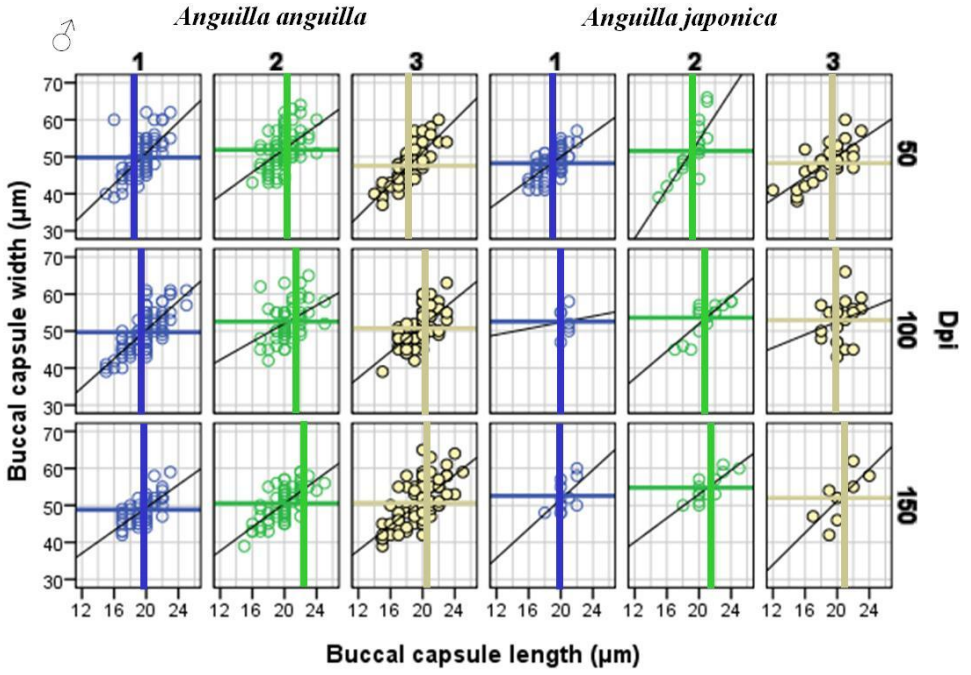

Figure 25: Buccal capsule length and buccal capsule width of male adults of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica* with the arithmetic mean values (horizontal and vertical lines). The black lines are linear regression lines. 1 – German parasite population, 2 - Polish parasite population, 3 – Taiwanese parasite population. Dpi – days post infection.

3.3.2 Hos-dependent morphological patterns

3.3.2.1 Body

The data referring to the body dimensions of the worms collected from the European eel was substantially more heterogenic and displayed a higher degree of dispersion than the data from the nematodes collected from the Japanese eel (Figures 22 - 27; Table 24). In the Japanese eel the worms were shorter and thinner (Boxes 25 - 26, 28 - 29; Figures 23 - 24; Table 24), had higher body length/width ratio that has not changed with time (Boxes 27, 30; Table 24) and have grown less quickly (Boxes 25 - 26, 28 - 29) than in the European eel.

Table 24: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the body (mm) of females and males of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica.*

Eel species	Worm sex	Measurements	N	Mean	SE	SD	Minimum	Maximum
A. anguilla	Female	Length	454	25.4	0.57	12.22	2.1	60.8
		Width	453	2.1	0.05	1.15	0.2	4.9
		Length/width ratio	453	13.2	0.19	4.14	6	60.8
	Male	Length	667	15.7	0.29	7.57	2.7	44.6
		Width	667	1.0	0.02	0.51	0.2	3.3
		Length/width ratio	667	16.3	0.15	3.92	6.3	45
A. japonica	Female	Length	161	16.3	0.74	9.45	3.5	46.5
		Width	160	1.1	0.06	0.76	0.2	3.1
		Length/width ratio	159	15.9	0.41	5.18	9	66
	Male	Length	194	8.8	0.31	4.39	2.2	25.1
		Width	195	0.5	0.02	0.32	0.2	1.7
		Length/width ratio	194	17.7	0.29	4.04	9.2	31.3

3.3.2.2 Oesophagus

No effect of eel species on the length of the oesophagus of the nematodes could be inferred but the oesophagus of the worms in the Japanese eel was thinner (Figures 24 - 25; Table 25) and grew less quickly (Boxes 31 - 32, 34 - 35), which suggests overall smaller dimensions of this body part in this eel species. In addition, the significance level of p=0.097 in case of the positive influence of the European eel on the oesophagus length allows an assumption of a trend directed towards a longer oesophagus in this eel species. The models revealed that in the Japanese eel the oesophagus length/width ratio was higher (Table 25; Boxes 33, 36), meaning a less compact shape.

Table 25: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the oesophagus (µm) of females and males of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica*.

Eel species	Worm sex	Measurements	N	Mean	SE	SD	Minimum	Maximum
A. anguilla	Female	Length	410	825.6	6.56	132.82	500.0	1170.0
		Width	409	272.6	3.86	77.98	26.0	700.0
		Length/width ratio	425	20.5	0.12	2.40	15.0	34.0
	Male	Length	632	722.6	4.30	108.01	420.0	1000.0
		Width	632	248.1	2.49	62.69	100.0	470.0
		Length/width ratio	629	19.7	0.08	2.02	14.0	26.0
A. japonica	Female	Length	157	747.6	10.01	125.47	490.0	1210.0
		Width	157	211.3	4.07	51.05	90.0	350.0
		Length/width ratio	155	20.6	0.20	2.45	15.0	27.0
	Male	Length	191	651.2	6.02	83.18	470.0	890.0
		Width	190	186.6	2.52	34.75	60.0	280.0
		Length/width ratio	193	19.7	0.15	2.09	12.0	25.0

3.3.2.3 Buccal capsule

The dimensions of the buccal capsule did not differ between the eel species (Boxes 37 - 38, 40 - 41; Table 26). In the Japanese eel the males had slightly but significantly lower buccal capsule length/width ratio suggesting a compacter body shape (Boxes 39, 42).

Table 26: The mean with standard error (SE), standard deviation (SD) and minimum and maximum values of the length, width with corresponding ratios of the buccal capsule (µm) of females and males of *Anguillicola crassus* in *Anguilla anguilla* and *Anguilla japonica*.

Eel species	Worm sex	Measurements	N	Mean	SE	SD	Minimum	Maximum
A. anguilla	Female	Length	425	20.5	0.12	2.40	15.0	34.0
		Width	425	54.6	0.30	6.09	40.0	74.0
		Length/width ratio	424	0.4	0.00	0.04	0.3	0.7
	Male	Length	629	19.7	0.08	2.02	14.0	26.0
		Width	632	50.5	0.22	5.51	37.0	74.0
		Length/width ratio	629	0.4	0.00	0.03	0.3	0.5
A. japonica	Female	Length	155	20.6	0.20	2.45	15.0	27.0
		Width	157	54.2	0.49	6.17	40.0	72.0
		Length/width ratio	155	0.4	0.00	0.03	0.2	0.5
	Male	Length	193	19.7	0.15	2.09	12.0	25.0
		Width	193	50.9	0.40	5.57	36.0	68.0
		Length/width ratio	193	0.4	0.00	0.03	0.3	0.5

3.3.3 Other effects

3.3.3.1 Density- and host-dependent regulation of worm size

Several interesting density-dependent associations among developmental stages coexisting within one swim bladder and the morphological traits exhibited by the adult worms could be determined: the more adults that were present in the swim bladder, the longer and wider were the body and oesophagus of the adults (Boxes 25 - 26, 28 - 29, 31 - 32, 34 - 35). Conversely, a higher number of adults had a negative effect on the length of the buccal capsule (Boxes 37 - 38, 40 - 41). Moreover, the more L3 that were present in the swim bladder wall, the shorter the body, oesophagus and buccal capsule (Boxes 25, 28, 31, 34), and the thinner the body and buccal capsule (Boxes 26, 29, 38, 41). In all cases there was a high degree of variability within the parameter values so that R^2 was low.
The models revealed no relationship between the eel length and the dimensions of the body, oesophagus or buccal capsule.

3.3.3.2 General morphological patterns

The models showed additionally morphological patterns that naturally occur in a developing population. The dimensions of the body and oesophagus and the length of the buccal capsule increased with the time and the body and oesophagus length/width ratios decreased meaning that the worms are growing more in the width than in the length (Boxes 25 - 36, 37, 40; Figures 22 - 25). Interestingly, the buccal capsule did not grow significantly wider with time suggesting that beginning from 50 dpi the differentiation of this metrical feature was fully completed (Boxes 38, 41; Figures 27 - 28). This was also confirmed by the increasing buccal capsule length/width ratio (Boxes 39, 42).

The models also revealed crucial differences between sexes of *A. crassus*. The males had a generally shorter and thinner body, oesophagus and buccal capsule and grew more slowly (Boxes 25 - 26, 28 - 29, 31 - 32, 34 - 35, 37 - 38, 40 - 41; Figures 22 - 27; Tables 24 - 26). In the European eel the length and width of the females exceed those of the males by a factor of 1.6 and 2.1, and in the Japanese eel by a factor of 1.85 and 2.2, for the length and width, respectively. The length/width body ratios were smaller for females in both eel species by a factor 1.05 and 1.3, in the European and the Japanese eel, respectively. For oesophagus, the length and width of this trait by the females exceed those by the males by a factor of 1.1 in the European eel and in the Japanese eel by a factor of 1.15 and 1.1, for the length and

width, respectively. The length/width oesophagus ratios were higher for females in both eel species by a factor 1.04.

In the European eel the length and width of the buccal capsule of the females were smaller than those of the males by a factor of 1.04 and 1.08, and in the Japanese eel by a factor of 1.04 and 1.2, for the length and width, respectively. The length/width buccal capsule ratios were the same for both sexes.

The higher length/width ratios of the body and buccal capsule of males suggest that they were less compact than the females and grew more in length and less in width (Boxes 27, 30, 39, 42). Conversely, the oesophagus length/width ratio for males was smaller (Boxes 33, 36) indicating a more compact shape.

4 Discussion

4.1 Divergence in recovery, developmental dynamics and reproductive potential

My common garden experiment revealed that populations of *A. crassus* have undergone genetic divergence in terms of infectivity (recovery) and developmental dynamics, while the reproductive potential was characterized by a high degree of phenotypic plasticity.

Recovery is a traditionally used parameter in experimental parasitology that describes the ability of the parasite to infect its host (Gandon et al., 1996). In the field, the prevalence (percentage of infected hosts) and mean intensity (mean number of parasites per infected host) are usually used to describe infection parameters. In my trial, the Taiwanese worms exhibited significantly higher recovery (35.5 – 44.4%) than the current European populations (13.8 – 30.3%) infecting the European eel. Apparently, my results mirror the infection dynamics observed in Europe beginning from the introduction event until the present time. After its introduction *A. crassus* became the most abundant helminth in the European eel (Sures et al., 1999). Later, after about a decade, the prevalence and mean intensities of infection had declined (Knopf, 2006). Since in the European eel no encapsulation processes could be verified and no acquired immunity was found (Knopf et al., 2008), the population density must have been regulated by some alternative means leading to a lowering of prevalence and intensities of infection in Europe. Density-dependent regulation of infrapopulation size in Europe (Fazio et al., 2008b), or thickening and sclerotization of the swim bladder wall lowering reinfection with the nematode (Würtz & Taraschewski, 2000) were proposed as possible mechanisms keeping the infection intensity at a lower level than directly after introduction. However, the decrease in infection parameters could also occur due to the co-adaptation of the parasite and its new host that has taken place during the 30 years after the introduction event. Considering that a single eel generation, which lasts up to 50 years (Tesch, 2003), may be infected with worms coming from 30 - 60 consecutive parasite generations, one must consider basic concept of the Red Queen hypothesis (Bell, 1982; Hamilton et al., 1990; Carius et al., 2001) which states that parasites, with their relatively short life cycles and large population sizes, evolve faster than their hosts. These in turn may buffer the effects of parasitism through genetic diversification of their offspring. Adaptation via the selection of less virulent genotypes of *A. crassus* could lead to a decrease in infection intensities, making them more tolerable to the host, as has been reported for other new host-parasite systems (Sasal et al., 1999; Mladineo & Maršić-Lučić, 2007). On the

other hand, natural selection of native-borne resistant genotypes of *A. anguilla* could also take place, but probably in a smaller extent, as only a part of European eel population that collectively spawn in the Sargasso sea is likely to be exposed to the selective pressure given by the parasite.

The data imply a genetically induced acceleration of development in the European worm populations. In both eel species for European nematodes the adult stage was reached earlier and more dead adults could be observed in earlier infection periods. While the first observation can simply be explained by accelerated moulting into adults, the latter seems to result from the earlier completion of the life cycle. The increase in developmental speed of the European populations can also be concluded from other parameters: in the European eel the number of European L4 larvae was higher early after infection and in the Japanese eel more dead larvae from the European populations could be observed. Interestingly, in experiments with European eels infected with European worms conducted in 2002 under similar conditions (at 23°C), no eggs were observed before 90 days post infection (Mahnke, 2002). In our trial, 5 years after that of Mahnke, the European nematodes reproduced prior to 50 days post infection suggesting that the life history evolution is directed towards progressively earlier reproduction and completion of the developmental cycle. Analogous trade-offs between decreased longevity and increased reproduction have already been reported for other free-living and parasitic organisms (Trouvé et al., 1998; Paterson & Barber, 2007).

The faster development of *A. crassus* in the European eel might be due to diverse selective forces acting on the parasite. As host immunity can restrain parasite multiplication (Hammerschmidt & Kurtz, 2005), the most important factor driving the divergence between populations seems to be the dissimilar immunological competence of the end hosts. In the European eel, the energy resources of the parasite originally used against the host's immunological onset became dispensable and could be relocated in faster completion of the developmental cycle. This is also supported by the fact that the slowest development was observed for the Taiwanese worms in their native host, where the effective immunological response of the Japanese eel is likely to constrain the parasite's developmental abilities.
However, one has to consider that the faster development of European populations might additionally or simultaneously result from an array of selective factors working at the environmental level. Divergence may depend on geographical variation, not host use (Morgan et al., 2005). Environmental temperature is probably one of the most important factors influencing the speed of development and can act as the main agent of selection (Fox

& Czesak, 2000; Campbell et al., 2001; David et al., 2003; Ayrinhac et al., 2004; Van Doorslaer et al., 2009). In colder climates, the life history cycle is usually accelerated because of the limited time available for reproduction (Addo-Bediako et al., 2002; Grapputo et al., 2005). Selection at lower temperatures may lead to the evolution of faster development, such as that demonstrated for *Drosophila melanogaster* (James & Partridge, 1995, 1998). Comparison of geographical populations in nine cosmopolitan species of *Drosophila* showed that temperate strains recovered faster from chill coma after cold treatment than tropical ones (Ayrinhac et al., 2004). Further, a number of insects have evolved differences in the duration of development, time at emergence, or diapause (Carroll et al., 1997; Byrne & Nichols, 1999; Groman & Pellmyr, 2000). The Russian population of Colorado potato beetles exhibits faster developmental rates at low temperatures than beetles from other European populations (Grapputo et al., 2005). Further, the population of pink salmon *Oncorhynchus gorbuscha* introduced to the European North of Russia exhibited changes in life history adapting it to colder water temperatures in the target area. The changes involved, among others, earlier anadromous migration of adult fish and faster maturation for successful spawning (Gordeeva & Salmenkova, 2011). In the natural range of *A. crassus* the water temperature is above 20°C most of the year (Mo & Steien, 1994). In Central Europe the temperature is seldom above 20°C with 8 months or more below 10°C (state on 04. 2011, http://www.lubw.baden–wuerttemberg.de/servlet/is/72252/). Accordingly, the European population might have responded to lower temperatures with evolution towards faster completion of the developmental cycle.

Moreover, the release from immunological stress in the European eel allowing increased infrapopulation densities could lead to the evolution of highly competitive genotypes (Wolfe, 2002; Colautti et al., 2004). Strong intraspecific competition (between individuals of the same developmental stage and between individuals of different developmental stages) for restricted living resources in an overcrowded swim bladder might lead to faster exploitation of a host, higher growth rates and evolutionary shortening of the life history duration (Trouvé et al., 1998) (see 4.2, 6.1 and 6.3). Thus, survivorship may be negatively affected by the density of conspecifics in one niche, as was shown for *Strongyloides ratti* (Paterson & Viney, 2002).

Finally, other factors, like the different quality of European waters, could also contribute to the observed changes in the parasite life cycle. Acidification, oil pollution or eutrophication may render hosts more susceptible to the parasites by reducing their immunological capabilities (Lafferty & Kuris, 1999; Sures & Knopf, 2004a).

4. Discussion

When applied back to the Japanese eel, the European parasites responded with a higher infectivity and faster development compared to the natural host-parasite system. However, these results have to be interpreted with caution as the differences in recovery occurred only in the early stages of infection and the differences in developmental dynamics were not consistent during the infection period. In this eel species, the most interesting difference between the parasite populations seems to be the higher number of encapsulated larvae from the European populations at 50 days post infection. Usually, after passage in the new host, the infectivity and the ability to develop in the former host should be reduced in comparison with the natural host-parasite system (Ebert, 1994; Blossey and Notzold, 1995). The higher larval mortality from the European populations observed in our trial could be due to attenuation created during passages in the new host as was shown for *Nippostrongylus brasiliensis* (Wescott & Todd, 1966). However, for other rodent models an enhanced virulence after passages in a new host has been reported (Dobson & Owen, 1977; Mackinnon et al., 2002).

On this place it has to be mentioned that the smaller differences between the parasite populations observed in the Japanese eel may be the result of the common garden experiment principles themselves. Some fitness components are only expressed under particular environmental conditions (Nuismer & Gandon, 2008). The water temperature of 23°C, which represents the optimal living temperature for the European eel, was chosen for my trail. For the Japanese eel the optimal temperature is around 28°C (Tesch, 2003). It is possible that at the higher temperatures which are common for the natural environment of the nematode, the differences between the nematode populations in the natural host would be more apparent.

As expected from previous findings in free living (Münderle, 2005) and experimentally infected (Knopf & Mahnke, 2004) eels, there are eel species-dependant effects on the infection dynamics of the parasites. The concept that population developmental dynamics differs in critical ways between populations in the native and introduced ranges of a species was proposed by (Elton, 2000). Differences in regulatory mechanisms, such as parasite-host relationships or inter- and intraspecific competition, may impose strong selective pressure on the life history traits of introduced populations. Life history changes can occur remarkably rapidly and have a substantial effect on population dynamics (Lambrinos, 2004). In my trail, the recovery of all parasite populations in the Japanese eel was lower than in the European eel. Lowered recovery in this eel species at 98 days post infection was previously reported by Knopf & Mahnke (2004), and our data suggests that this difference resulted mainly from reciprocal dynamics between the L4 and encapsulated larvae (higher number and faster

decrease of L4 larvae and simultaneous higher number and faster increase of dead larvae). This finding suggests that the immune response in the Japanese eel acts predominantly on L4 larvae and the switch from L4 larval stage to adult is the most important developmental event in the natural host-parasite system. Hence, the nematodes exhibited altered developmental patterns when harboured in different end-hosts. In the European eel the number of L3 and L4 larvae decreased, but the number of living and dead adult worms increased in the course of infection resulting in higher numbers of these developmental stages in this eel species. These developmental dynamics may result from undisturbed moulting to the next life history stages in the immunologically naive colonized end-host. In comparison, in the Japanese eel the numbers of L3 and L4 larvae and adults decreased, but the numbers of dead adult worms and larvae increased with time. This effect can be interpreted as a developmental slow down caused by the effective immune response of the native host being expressed predominantly in the decreasing number of adults. Similar host-dependant infrapopulation composition was previously reported by Knopf & Mahnke (2004). However, my results are partially in contradiction with those of Knopf & Mahnke (2004) who found higher numbers of larval stages in the Japanese eel 98 days post infection, concluding slower development of the nematode in this eel species compared with the European eel. My data displayed overall lower numbers of L3 larvae and a faster decrease of L4 larvae in the Japanese eel, which suggests faster development of the nematodes in this eel species relative to the European eel.

My study revealed that all parasite populations infecting the European eel reproduced more effectively than in the Japanese eel suggesting that egg production is regulated by phenotypic plasticity. The lower reproductive output in the Japanese eel is also in agreement with previous investigations by Knopf & Mahnke (2004), who showed that at 98 days post infection the German population of *A. crassus* reproduced successfully in 88% of experimentally infected European eels and only in 2% of the Japanese eels. For free-living or parasitic platyhelminthes, the total reproductive capacity was found to be directly determined by the size of the worm (Trouvé et al., 1998). It is known from the former studies (Knopf & Mahnke, 2004; Münderle, 2005), as well as from my trial (see 4.2), that *A. crassus* grows to a larger size in the European than in the Japanese eel. I also found that the number and length of adults has a positive effect on the number of eggs. The higher reproductive potential in the European eel may thus represent a response pattern typical for multicellular parasites in which increased host exploitation involves a greater conversion of host tissues into parasite tissues and parasite eggs (Ewald, 1995) and the increased density of adults within one niche enhances the intraspecific contact making the chance of finding a mate more likely (Rohde,

1991). A plastically regulated increase in fecundity was also reported for an introduced population of pink salmon, *O. gorbuscha*, which is believed to be connected to the higher body weight of the fish compared to the source population (Gordeeva & Salmenkova, 2011).

4.2 Divergence in morphology

My study revealed that the body, oesophagus and buccal capsule of *A. crassus* are independent traits and both the plastic and genetic responses were probably involved in the morphological variation observed among populations of the nematode. When infecting the European eel, all parasite populations had equally larger body sizes and grew more quickly than in the Japanese eel, but under the same living conditions (in the same eel species) no differences between the nematode strains could be revealed (Figure 28). This suggests that the parasites have responded plastically to the new environment in the European eel and the increase in body dimensions is a phenotypic modification. The same is true for the body shape as no differences in the body length/width ratio between the nematode populations harboured in the European eel could be detected.

In turn, the dimensions of the oesophagus were larger, the shape compacter and the growth more rapid for all three parasite populations in the European eel, but when infecting the same eel species (both in the European or in the Japanese eel) the oesophagus of the Polish population was larger and compacter than in the German and Taiwanese populations (Figure 28). This indicates that both the phenotypic plasticity and genetic adaptation have shaped the dimensions of this morphological trait.

The phenotype of the buccal capsule was identically expressed by all parasite populations independently of the infected eel species, which excludes the involvement of phenotypic plasticity in the observed morphological variation of this trait. When infecting the same eel species the Polish population had the biggest buccal capsule (similarly to the oesophagus), but the buccal capsule of the German population was smaller and less compact than that of the Taiwanese population (Figure 28). The shape of this trait did not differ between the Polish and Taiwanese populations but both populations had a compacter buccal capsule than the German population. Again, both European populations appeared to be different from one another, in this case, in terms of buccal capsule morphology.

A compilation of the morphological differences between the *A. crassus* populations under the same (in *A. anguilla* or in *A. japonica*) or different living conditions (in *A. anguilla* and in *A. japonica*) are presented in the figure 28.

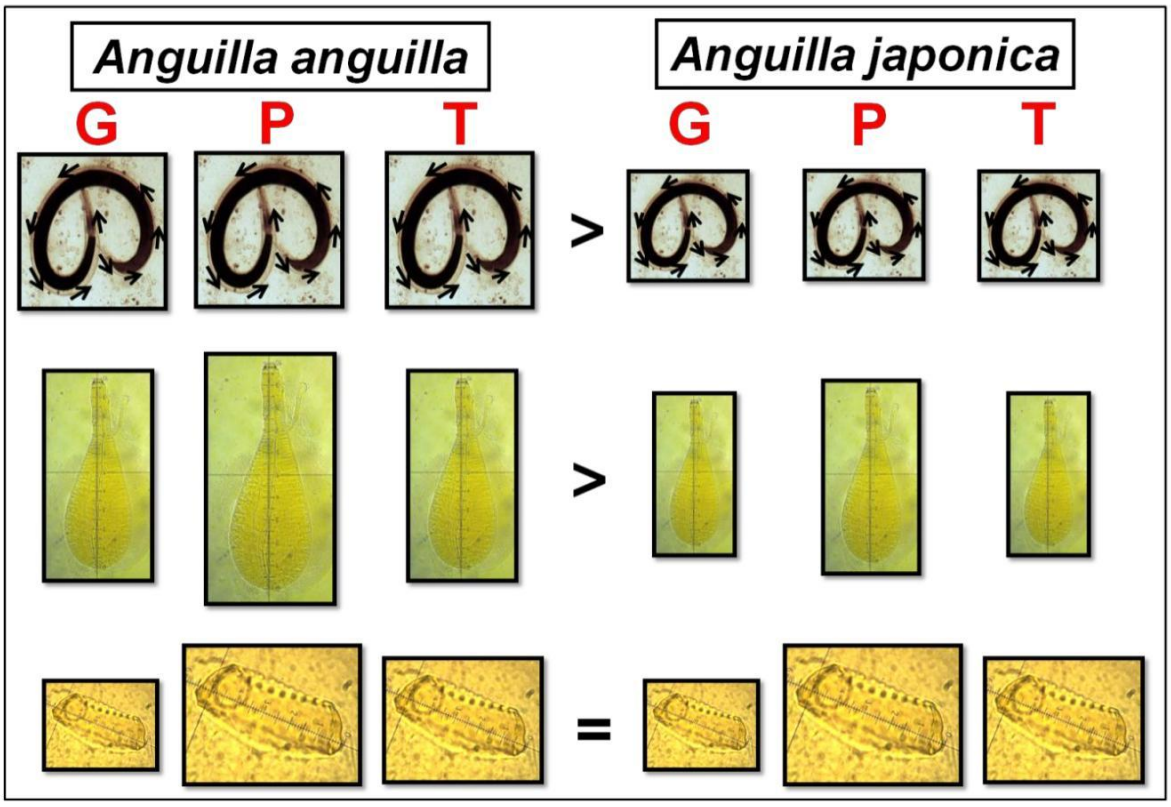

Figure 26: Schematic presentation of the plastic and genetic responses expressed by *Anguillicola crassus* populations infecting the European (*Anguilla anguilla*) or/and the Japanese eel (*Anguilla japonica*). G – German parasite population, P – Polish parasite population, T – Taiwanese parasite population.

After invasion the invading taxa often undergo changes in body size and shape in comparison to their native range, becoming either larger or smaller. With the exception of the marine invasive invertebrates that exhibit a clear directional pattern becoming larger in the colonized range relative to their native range (Grosholz & Ruiz, 2003), studies of plants (Thébaud & Simberloff, 2001), lizards (Losos et al., 1997), mammals (Dayan and Simberloff, 1994) and birds (Johnston & Selander, 1973) shown that no general pattern of change can be assumed.

Enlargement of the morphological features of *A. crassus* collected from the wild European eels compared with the worms found in the wild Japanese eels were previously reported by Münderle (2005). Also an experimental study given by Knopf & Mahnke (2004) showed that the worms recovered from the European eels experimentally infected with European larvae were heavier than the European worms recovered from the Japanese eels.

In the field study of Münderle (2005), amongst others, the German population of *A. crassus* from the European eel was compared in terms of morphology of the body, oesophagus and buccal capsule with (i) the Polish parasite population collected from this eel species and (ii) the Taiwanese population collected from the Japanese eel. That study revealed no

differences in body length and width between the German and Polish parasite populations infecting the European eel, which is in agreement with my observations. However, the differences in terms of body ratio between the Polish and German populations reported in that study are contradictory with my results. Further, similarly to my results (the German parasite population in the European eel vs. Taiwanese parasite population in the Japanese eel), the Taiwanese worms from the wild native host were smaller than the German parasite population collected from the European eel (Münderle, 2005). Finally, I found that the Taiwanese population in the Japanese eel had smaller body length/width ratio i.e. compacter body shape confirming the field data of the Münderle (2005). This result definitely distinguishes the body shape of *A. crassus* in its natural host from all other host-parasite combinations. However, as all nematode strains in the European eel were still the same in terms of body shape, the evolution of this trait cannot be assumed.

Larger dimensions and higher growth rates of the oesophagus expressed by all parasite populations infecting the European eel compared with parasites recovered from the Japanese eel also find a confirmation in the study carried out by Münderle (2005), suggesting a high plasticity of this feature. Nevertheless, the author found no differences between the Polish and German parasites infecting the colonized host while my results showed larger oesophagus size of the Polish parasites in both eel species. However, in the study of Münderle (2005) a genetic divergence in oesophagus was shown to occur between the German population from the Rhine River near Karlsruhe (analogous to the sampling place chosen for the presented study) and three other European populations (Rhein/St. Goar, Plauer See and Ostsee/Stockholm). This is rather surprising, as the differences with respect to pharynx size between the German populations would be bigger than between the German and Polish parasite strains. It is possible that the genotypes linked to this morphological feature chosen for that study were strongly affected by the sampling, with distinctive genotypes being present only at particular location.

A very interesting pattern of genetic divergence was displayed by the buccal capsule. As no differences between populations were revealed when infecting different eel species, the buccal capsule seems to be the most appropriate morphological feature for measuring the evolutionary change. The Polish worm population had larger buccal capsule than the German and Taiwanese populations when harboured both in the European and the Japanese eel hosts but, surprisingly, the German parasite population has evolved a smaller buccal capsule than its Taiwanese conspecifics (Figure 28). Also Münderle (2005) in his field study found that the nematode population originating from the Polish eels evolved bigger buccal capsules compared with the German parasite population sampled in the Rhine River, giving the results of this study a strong support. However, for the comparison between

naturally occurring German (from the European eel) and Taiwanese (from the Japanese eel) parasite populations contradictory results were revealed, showing larger dimensions of the buccal capsule in the German strain in its colonized host comparing with the natural host-parasite system.

The larger size and higher growth rates of the body and oesophagus in all populations of *A. crassus* infecting the European eel represent a plastic response to new living conditions in the European eel. Such phenotypic plasticity is a characteristic of many invasive species allowing immediate adjustment to different habitats (Sakai et al., 2001; Allendorf and Lundquist, 2003). For other parasitic worms that have been maintained in different hosts, strong morphological changes induced by phenotypic plasticity have been reported, for example in *Schistosoma mansoni* adult male worms which are bigger in *Rattus rattus* than in *R. norvegicus*, as well as in *Nectomys squamipes* compared to Swiss Webster (SW) mice (Neves et al., 2004). There is a range of possible environmental cues that could trigger the plastic responses of the original genotypes of *A. crassus*.

First, in the context of a newly colonized host in which immunological stress is lacking, individuals that do not allocate resources to defence may have a greater fitness in terms of reproduction than those that do (Keane & Crawley, 2002). In addition, larger body size is believed to increase overall fitness (Kingsolver & Pfennig, 2004). Host immunity was shown to restrain parasite growth (Hammerschmidt & Kurtz, 2005). Accordingly, the energy won by *A. crassus* through immunological release in the colonized European eel was probably relocated in intensive blood intake and ingestion resulting in higher growth rates of the body and oesophagus. Phenotypic plasticity induced by differences in food resources is one of the most likely factors explaining differences in body mass and size of the populations of Mediterranean blue tits *Cyanistes caeruleus*, with higher mass and larger size being expressed in the population exposed to better nutritional conditions (Blondel et al., 2006). A food-dependent size variation in experimentally introduced brown anole *Anolis sagrei* lizards was also induced by the phenptypic plasticity (Campbell & Echternacht, 2003). Differences in nutritional value among different host-plant species are believed to be translated into plastically induced changes in the body size of their phytophagus insects (Diegisser et al., 2007). Further, body size (length and weight) of the copepod *Calanus finmarchicus* exposed to various thermal and nutrition conditions was positively related to food concentration, but inversely related to temperature (Campbell et al., 2001).

Thus, temperature might also be an environmental factor shaping morphological variation. As described above (see 4.1), in the natural range of *A. crassus* the water temperature is above 20°C most of the year (Mo & Steien, 1994). In Central Europe the temperature is seldom

above 20°C with 8 months or more below 10°C. Accordingly, the plastic changes in parasite dimensions might have been induced by lower temperatures. The population of pink salmon *O. gorbuscha* introduced to the European North of Russia have responded plastically to colder environmental conditions with more rapid growth resulting in higher body mass (Gordeeva & Salmenkova, 2011). However, higher growth rates of the lizard *Sceloporus undulates* are assumed to be of genetic origin linked to the warmer environment serving as an ecological source of variation (Niewiarowski & Roosenburg, 1993). Also populations of the European wild rabbit *Oryctolagus cuniculus* introduced into Australia in 1859 evolved leaner bodies and longer ears in response to warmer climatic conditions. This morphological cline resulted from both a genetic and plastic response (Williams & Moore, 1989a).

In the general, populations exposed to new climatic gradients often evolve clinal variation in traits such as body size (Baker, 1980; Huey et al., 2000). In many species, high latitude populations are substantially larger than their low latitude equivalent. Studies with *Drosophilla melanogaster* have shown that large body size may be adaptive at low temperatures (Reeve et al., 2000). Further, changes in morphological traits (wing size) of malaria vector populations of *Anopheles funestus* in Cameroon appear to be under natural selection and contribute to local adaptation. This variation was found to be dependent on temperature and elevation with larger dimensions expressed at lower temperatures (Ayala et al., 2011). However, no significant impact of latitude was revealed for other species including the crab *Carcinus minus,* for which a negative relationship with latitude was found (Atkinson & Sibly, 1997).

The next selective pressure that might have forced the plastic increase in growth rates and dimensions of the body and oesophagus is intraspecific competition. Münderle (2005) in his field study found a positive correlation between the number of worms and their body mass, which may indicate that the worm size has been positively influenced by the increased infrapopulation density. This association is rather unusual as for many host-parasite systems a crowding effect was postulated, such that the size of worms in an infection is inversely proportional to the number of worms within a host (Heins et al., 2002). My analysis of density-dependent regulation of worm size also failed to reveal a positive result (see 6.3). Assuming the a crowded swim bladder offers only limited food supplies, the nematodes would adapt by competing for obtaining host resources as quickly as possible, which may result in the observed elevation of growth rates and morphological dimensions (Mideo, 2009). The latter may in turn induce faster completion of the life cycle that would trade-off with the higher reproductive output (see 4.1). However, whether the limited food resources in the immunologically naive European eels constituted a selective pressure for the stronger competition remains an open question.

4. Discussion

In the context of evolution, contrary to the body size, the oesophagus and buccal capsule present interesting morphological features. The dimensions of pharynx were larger and the shape compacter for all three parasite populations in the European eel, but when infecting the same eel species (both in the European and in the Japanese eel) the oesophagus of the Polish population was larger and compacter than in case of the German and Taiwanese populations. This indicates that oesophagus variation may depend on the genetic and plastic responses.

However, the differences in the buccal capsule seem to be a product of genetic divergence alone, as no differences between populations could be found when infecting different eel species. The Polish worm strain had a larger buccal capsule than the German and Taiwanese populations when harboured both in the European and the Japanese host, but surprisingly, the German parasite population evolved smaller and less compact buccal capsule than the Polish and Taiwanese parasite populations. Morphological traits that are subject to genetic selection often target a defined functional affiliation and changes in their morphology are a product of genetic adaptation resulting in evolutionary divergence with respect to these traits. Both oesophagus and buccal capsule are specialized, the former is a muscular pump directly involved in food (blood) uptake (Bruňanská et al., 2007) and the latter is used to anchor the worm to the swim bladder tissue (Bruňanská et al., 2010). Genetic adaptations of functional traits leading to an increase in fitness are provided by a number of studies. The native Australian snakes that are endangered by an invasive cane toad *Bufo marinus* have evolved a morphologically smaller head size preventing them from swallowing bigger toads (Phillips & Shine, 2006). The hind limb length of *Anolis* lizards introduced to several small islands has evolved as a respond to changes in vegetation structure (Losos et al., 1997). Soapberry bugs *Jadera haematoloma* that colonized three new species of introduced plants in North America over the past century (Carroll & Boyd, 1992) exhibit repeated adaptive changes in the beak length that have a genetic basis (Carroll et al., 1997). For other phytophagus insects adaptive differences in mandibular surface, mandibular width or ovipositor characters were postulated (Pappers et al., 2002; Diegisser et al., 2007), and marine snails have evolved a morphological adaptation in anti-predator armour (Seeley, 1986). However, intraspecific variation in functional traits induced by the environment has also been reported. Numerous invasive species may be capable of quickly modifying their feeding apparatus to overcome prey resistance (Smith, 2004). For *Schistosoma mansoni*, a rapid change in morphology of suckers and genital system was observed as response to different living conditions (Neves et al., 2004).

A. crassus has undergone evolutionary divergence with respect to infectivity, developmental dynamics and morphology of oesophagus and buccal capsule within a maximum time span of 30 years, equivalent to about 30 - 60 generations of the parasite in the European eel.
The available literature suggests that evolutionary changes in introduced species can occur remarkably rapidly (Campbell & Echternacht, 2003). Referring to Reznick & Glambour (2001) an adaptively rapid evolution should take place at a time scale observable by the investigator. The introduced small Indian mongoose, *Herpestes auropunctatus*, which was originally allopatric with the larger congeners the grey mongoose *H. edwardsii* and/or the ruddy mongoose *H. smithii*, evolved larger skull length and larger canine diameter within several decades in areas where these species are sympatric (Thulin et al., 2006). Dlugosch & Parker (2008) reported the evolution of growth rate and phenology of *Hypericum canariense* which occurred within 50 years (25 generations). A common garden experiment on populations of the butterfly *Pararge aegeria* revealed that the colonizing population responded with enlargement of wings and thorax within 20 years (Van Dyck et al., 1998), and Hendry et al. (2000) provided evidence of rapid evolution in introduced salmon that has taken place in only 13 generations.

The available literature suggests that the majority of rapid evolutionary changes are a response to anthropogenic changes often connected with colonization process (Reznick & Ghalambor, 2001). Morphological traits that rapidly evolved include changes in body size in birds, fish and flies (Baker, 1980; Reznick et al., 1990; Huey et al., 2000) or feeding morphology in bugs and birds (Carroll & Boyd, 1992; Smith et al., 1995). Physiological characters included salinity tolerance in copepods (Lee, 1999), local adaptation of bugs to the host's chemistry (Carroll et al., 1998), heavy metal tolerance in plants and animals (MacNair, 1987; Klerks & Weis, 1987), insecticide resistance (Rosenheim et al., 1996), and thermal tolerance in fish (Holland et al., 1974; Hendry et al., 1998).

4.3 Phenotypic plasticity vs. genetic divergence

Patterns of life-history and morphological variation have often been interpreted with regard to their evolutionary importance (Pigliucci, 2005; Laffont et al., 2010). However, they may also respond to environmental variation in complex ways, suggesting that the range of phenotypes produced by a particular genotype might be different depending on environmental conditions. As a result, any variability may be part of a plastic or an adaptive response (Carreira et al., 2006). For this reason, investigations on life-history and

morphological traits necessarily require the simultaneous analysis of genetic and environmental factors, which may shape intraspecific variation (Mackay, 2004).

After the introduction event, the elevated infection intensities of *A. crassus* coincided with an increase in growth rate, enlargement of the body and oesophagus dimensions and an increase in reproductive output. Agrawal et al. (2002) and Sexton et al. (2002) postulated that phenotypic plasticity plays a crucial role during establishment, allowing species to expand rapidly across diverse landscapes. Later, selection should favour local adaptation, and this should lead to an increase in local fitness. The extensive nutritional exploitation of the immunologically naive European eel by the nematode allowed a greater conversion of host tissue into parasite tissues and thus higher eggs production. Thus, the plastic enlargement of the body and oesophagus, as well as elevated reproductive output, may present a colonization strategy of the nematode providing for successful dissemination. The rapid population growth was additionally facilitated by high infection intensities following introduction. After the successful establishment and colonization of the parasite population in the European eel host, genetic selection for lower infectivity and faster development in both European populations, larger oesophagus and buccal capsule in the Polish parasite population and smaller buccal capsule in the German parasite population compared with the Taiwanese parasite strain has taken place. Figure 29 presents a theoretical scenario of changes that could have taken place after the introduction of *A. crassus* to Europe, with consideration of parameters of infection dynamics reported for the European eel (for details see 1.2.2 and 4.1).

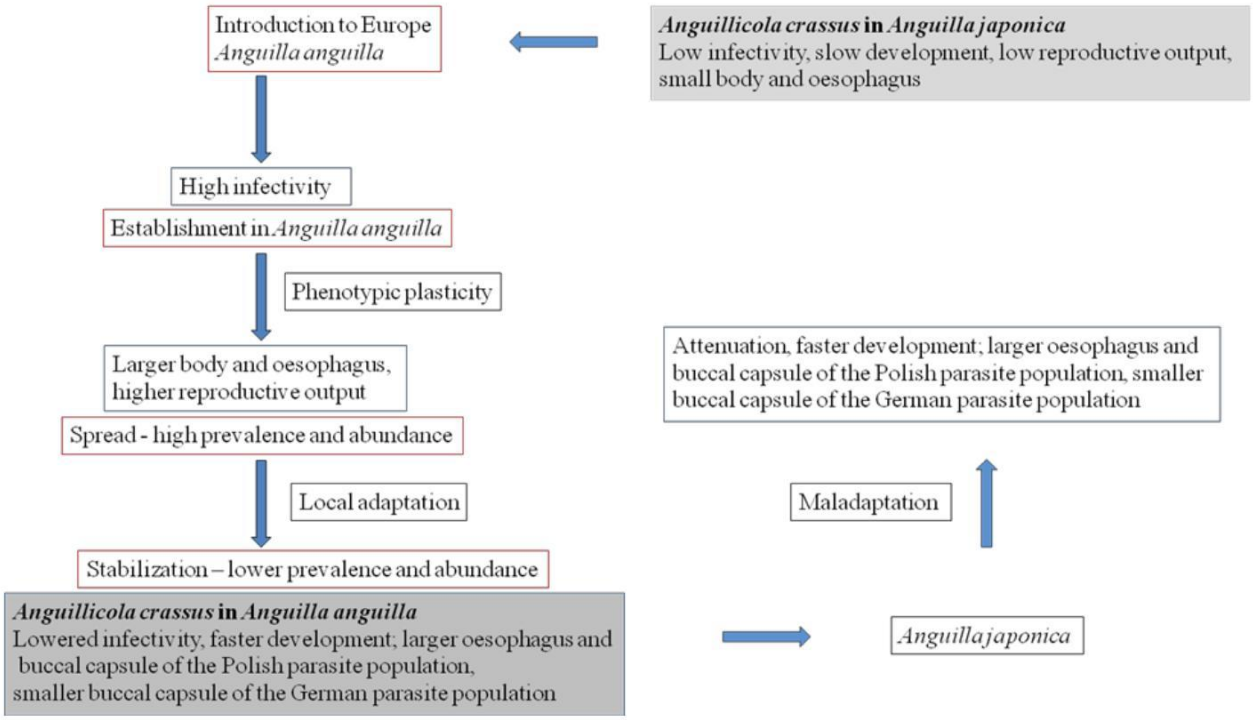

Figure 27: On the right: a possible scenario of the divergence of *Anguillicola crassus* in Europe based on the common garden experiment with the German, Polish and Taiwanese parasite populations infecting the European eel (*Anguilla anguilla*) in relation to changes in infection parameters reported for the eel population in Europe. On the left: genetic changes observed in the current Polish and German nematode populations after a back transfer to the Japanese eel (*Anguilla japonica*) relative to the natural host-parasite system (Taiwanese parasite population - *Anguilla japonica*).

Traditionally, in a host-parasite systems local adaptation should occur, when parasites, conditioned by a greater dispersal ability, show more frequent reproduction and/or higher virulence and have a higher performance (infectivity, recovery) on their local host compared with their foreign host (Kaltz & Shykoff, 1998; Greischar & Koskella, 2007). This has been shown in several cross infection experiments, e.g. for trematodes *Microphallus* sp. in the snail *Potamopyrgus antipodarum*, *Schistosoma mansoni* in *Biomphalaria* sp., *Diplostomum phoxini* in the fish *Phoxinus phoxinus* or the microsporidian *Pleistophora intestinalis* in *Daphnia magna* (Gandon et al., 1996). However, Kaltz and Shykoff (1998) distinguished between the process and pattern of adaptation. The pattern of adaptation compares performance in local hosts with that in foreign hosts, while the process of adaptation deals with the mean fitness of parasite populations before and after the selective response within their host populations. In the case of the pattern of adaptation there is no general rule of performance of the parasite population on the new host (adaptive traits present in the native host may allow increased performance, leave performance unchanged or diminish it), whereas the process of adaptation should result in the higher fitness of the established population in comparison with the state before establishment. In terms of the pattern of

adaptation, all three *A. crassus* populations in European eel expressed higher performance than in its native host. In my trial the process of adaptation refers to the European parasite populations in relation to the Taiwanese population infecting the European eel. If it is assumed that higher infectivity is a measure of fitness, *A. crassus* in Europe would be maladapted to the European eel, as the performance on the local host decreased. However, in the light of fitness related traits, the decreased infectivity may be beneficial for the parasite, as it may prevent the over-exploitation of the host and lead to overall better survival of the parasite (Sicard et al., 2007). Moreover, if it is assumed that infectivity is linked to virulence, the decrease in infection intensities may be adaptive and understood in the framework of inclusive fitness theory (Wild et al., 2009). In this regard, the role of reciprocal immunological coadaptation of the European eel to the new parasite contributing to decrease of infectivity should not be overlooked (Mideo, 2009).

Further, from the classical point of view, the higher egg production would not be classified as important in adaptive evolution, as selection that acts on non-heritable phenotypic variation does not produce an evolutionary response (Endler, 1986). However, modern evolutionary biology states that plasticity should be a subject to selection itself (Ghalambor et al., 2007). Moreover, plastic responses should also be adaptive (in the evolutionary sense of improving the organism's survival or reproduction), playing an important role in creating the conditions for selection to act on which consequently may cause an adaptive genetic response (genetic assimilation) (West-Eberhard, 2005). Holding the view, adaptive plasticity would be not only lead to the higher reproductive output, but also the larger body and oesophagus dimensions. The adaptiveness of the genetically fixed larger dimensions of the oesophagus and buccal capsule in the Polish parasite population and smaller dimensions of the buccal capsule observed in the German strain should be seen in their improved functionality to blood intake. However, any selective forces leading to such morphological adaptations are unknown and their adaptive character is only speculative.

Finally, the adaptive role of faster development evolved by the parasites in the European eel may lie in the earlier reproduction required in the colder water bodies in the new area. Such adaptations to cold climatic conditions may help the nematode to invade areas further to the north of their current distribution, for example Iceland, where populations of *A. crassus* are not present (Jakob et al., 2009b). Alternatively, the decreased longevity of the European population may represent a trade-off with increased reproductive potential (see 4.1).

In the light of evolution, the buccal capsule seems to be the most appropriate morphological trait studied here as it is used as a taxonomic marker for distinguishing among these parasites on the generic level (Moravec, 1994). As this morphological feature is thought to be

differentiated very early in post embryonic development, the changes in its dimensions should occur only to a minimal extent during the life span of the parasite and should be independent of the dimensions of the body or the oesophagus. This special character of the buccal capsule was also confirmed in my study. While the body and oesophagus were growing during the infection period, the dimensions of the buccal capsule, particularly of its width, did not change beginning from 50 dpi. This highlights the buccal capsule as a feature that is peculiarly appropriate for the investigation of evolutionary changes, as the time factor as well as other secondary factors influencing the growth rate can be excluded in the interpretation. If evidence of evolution only relies on the differences in the buccal capsule, then genetic divergence would have taken place between all investigated parasite populations. Assuming no gene flow between the parasite strains, divergent evolution and potentially speciation of the European populations would be imaginable.

Finally, it has to be emphasized that the whole array of observed intraspecific variability in *A. crassus* is determined by the genetic response that may result from the lowered genetic diversity in European populations due to a bottleneck after introduction (Wielgoss et al. 2008), founder effect (Dlugosch & Parker, 2008), a random genetic drift (Kolbe et al., 2008) (especially in case of the morphological divergence between both European populations), or even a simple sampling artefact, which are also strong evolutionary forces (Thulin et al., 2006). Nevertheless, whatever the genetic bases or selective pressures inducing the intraspecific variation, my study reveals that *A. crassus* is undergoing genetic divergence in ecological time due to 30 years of spatial isolation.

4.4 Regulation of infrapopulation density and worm size

The regulation of parasite infrapopulation size should prevent an over-exploiting of the host and lead to stability of a host-parasite system in time and space (Anderson, 1991). As one host offers only limited food resources, the regulation of infrapopulation structure should be regulated by the size of the host (living niche), the size of the parasite should depend of the infrapopulation density and the infection dynamics should be self-regulated by the infrapopulation itself (Ashworth & Kennedy, 1999; Fazio et al., 2008b). Moreover, the regulation of parasite infrapopulations should depend on other factors, such as the mode of parasitism (ecto- or endoparasites) or the immunological competence of the hosts (Anderson & May, 1978; Keymer, 1982; Sire et al., 1998; Andreassen et al., 1999; Roberts, 2000; Paterson & Viney, 2002; Sorci et al., 2003; Bush & Lotz, 2009).

4.4.1 Self-regulation of infrapopulation density

The self-regulation of infrapopulation density refers to the interaction between various developmental stages that shape the developmental dynamics within one living niche (host).
My study revealed a new set of regulative relationships between developmental stages coexisting in one swim bladder that have not been reported before. On the one hand, I found that the increasing numbers of L4, adults and eggs positively influences the density of L3 in the swim bladder wall, which implies that with enhanced density of those developmental stages, the migrating L3 larvae are allowed to enter the swim bladder wall. This suggests that not all L3 larval stages were present in the swim bladder wall at the time of investigation. The migration route of the L3 larvae leads through the digestive tract near the liver into the swim bladder (Moravec et al., 1994). However, I found no L3 larvae either in the stomach or in the gut of the eels. It is possible that the L3 larval stages reside in other organs, as they have also been observed in muscular tissue, liver or kidney (Haenen et al., 1989, 1994b; Van Banning & Haenen, 1990). On the other hand, I found that the entrance of L3 larvae into swim bladder wall was limited by the presence of fully grown adult worms.

Fazio et al. (2008b) found that moulting to the L4 larval stage is accelerated by an increased number of L3 larvae. This result is in contradiction to investigations of Haenen et al. (1996), but is confirmed by my study that revealed that the density of L4 larvae was enhanced with an increased number of L3 larval stages. Additionally, my data suggests a positive influence of the adults on the number of dead adults implying that developmental speed may be dependent of the higher adult density within one swim bladder.
Ashworth and Kennedy (1999) postulated that an increased number of adults retards the development of larvae to adults indicating an over-crowding effect. Later Fazio et al. (2008b) found that the moulting of L4 to adults is constrained by the male worms but enhanced by the opposite sex. My study did not confirm either of these findings as no influence of adults on the L4 larval stage was found.
In the study mentioned above, Ashworth and Kennedy (1999) postulated that increased numbers of adults constrain reproductive capacity (measured as the number of fertile females). Referring to this fitness component, I came to other results which show that reproductive success increased with higher numbers of adults, dead adults and the length of the adult worms. These associations seem to be plausible, as a higher density of larger adults in a swim bladder enhances the chance of mate (Rohde, 1991; Trouvé et al., 1998) and multiplies the number of eggs laid. However, this is not universal for all host-parasite systems. In a study on density-dependent fecundity of *Schistosoma mansoni* in humans or

Strongyloides ratti in rats, a negative relationship was found between the numbers of eggs and the total number of worms (Medley & Anderson, 1985; Paterson & Viney, 2002).
Further, Fazio et al. (2008b) found that the number of eggs positively influences the number of L3 suggesting that with progressive reproduction (presence of eggs) the next L3 are allowed to enter the swim bladder. The positive influence of number of eggs on the density of adults recovered alive and dead adults revealed in my study suggests a similar effect that would state that when the reproduction was underway (presence of eggs) the more larvae were allowed to mature and reproduce and the more worms died after completing their life history. The last association was more pronounced in the Japanese eel than in the European eel.

As the infrapopulation self-regulation mechanisms were in most cases analogous for both eel species, the thesis proposed by Fazio et al. (2008b), stating that the decline of the infection intensities observed in Europe has been due to regulation of infrapopulation density, has to be negated. The only difference between the eel species in the regulatory mechanisms was conveyed in the more pronounced reciprocal negative association between the dead larvae and dead adults detected in the Japanese eel. As the underestimated presence of the dead larvae in the European eel cannot have any significance for any regulatory process in this eel species, and the all others interactions were overlapping for both final host species, the decrease of infection intensities observed in Europe are more likely to have arisen in the process of local adaptation/coadaptation. The pronounced influence of the number of eggs on the performance of adults observed in the Japanese eel does not seem to constitute a crucial mechanism differentiating both eel species in terms of regulation of infrapopulation density from one another.

Concluding, any significant association between the performance of a particular life history stage was directed towards the positive influence between them, generally indicating an acceleration in the development due to increased density of any developmental stage. This result is important in regards to faster development of the European worms which was proposed to be linked to intraspecific competition forced by the increased infrapopulation density observed especially in the European eel (see 4.1).

4.4.2 Host-dependent regulation of infrapopulation density

Several authors have argued that the density of an infrapopulation of *A. crassus* in the swim bladder of an eel is constrained by space: high numbers of parasites were found in larger

swim bladders, while low numbers were usually found in smaller swim bladders (Van Banning & Haenen, 1990; Ashworth & Kennedy, 1999; Lefebvre et al., 2002a; b; Palstra et al., 2007; Fazio et al., 2008b). My results did not confirm this relationship, as no influence of the size of the eel on the total recovery or the intensity of any of the developmental stages could be revealed. This result is in agreement with Münderle (2005) who found no effect of the length of the wild European eels or the swim bladder length on the number of adult worms of *A. crassus*.

4.4.3 Density-dependent regulation of worm size

The regulative role of infrapopulation density on the size of the worms has been documented for many macroparasites. Again, for plerocercoids of *S. solidus* and the three-spined stickleback, crowding effects were postulated, meaning that the dimensions of the parasites are regulated by their number, i.e. the more parasites coexist within one host, the smaller are their bodies (Heins et al., 2002). For the tapeworm *Hymenolepis diminuta* a negative relation between the number of worms and their length was revealed (Lowrie et al., 2004). Also for nematodes, density-dependent size regulation has been reported: in the case of *Protospirura muricola* that lives in the stomach of a spiny mouse *Acomys dimidiatus*, the mass and the length of the worms decreased with the increasing number of the parasites (Lowrie et al., 2004). However, Münderle (2005) in his field study found no relationship between the number of worms of *A. crassus* and the dimensions of their body, oesophagus and buccal capsule or even a positive correlation between the number of worms and their body mass. Nevertheless, as *A. crassus* in the eel represents a host-parasite system in which various developmental stages coexist within one swim bladder, I checked for influence of the intensity of particular life history stages (separately for L3, L4 and adults) on the dimensions of the body, oesophagus and buccal capsule of the adult worms. The data revealed a negative effect of the number of adults on the length of the buccal capsule, meaning that this dimension decreased with increasing number of adults. This association found a confirmation in case of the German parasite population where, in relation to the Taiwanese population, higher numbers of adults hold the smallest buccal capsule. However, it cannot be seen as a rule, considering that at the same adult densities the Polish parasite strain expressed the biggest buccal capsule.

Further, the size of the adults, their oesophagus and buccal capsule should depend negatively on the number of L3 larvae in the swim bladder wall. In the light of density-dependent regulation of worm size my study showed that larval stages also play a role in

density-dependent regulation of worm size and that different morphological traits can be regulated by different developmental stages.

4.4.4 Host-dependent regulation of worm size

The idea that the dimensions of the parasite are influenced by the size of its host was already hypothesised at the beginning of the past century (Harrison, 1915). A positive relationship between the growth of the host and its parasite was shown, e.g. for plerocercoids of *Schistocephalus solidus*, a cestode that uses the three- spined stickleback, *Gasterosteus aculeatus*, as its second intermediate host (Barber, 2005). Fazio et al. (2008b) found that the weight of adult *A. crassus* should be constrained by the size of the swim bladder of the European eel. Oppositely, Münderle (2005) in his field study with *A. crassus* and the wild European eels did not find any correlations between dimensions of the body, oesophagus or buccal capsule with the length of the final host or the length of the swim bladder. I also did not find any influence of the length of either European or the Japanese eel on the morphology of these traits. Thus, for *A. crassus*, independently of which host is parasitized, the restriction of parasite growth by the size of the final host cannot be confirmed. The size of the parasites seems to be regulated by the presence (the Japanese eel) or the absence (the European eel) of the immunological response of the host (Nielsen, 1999).

5 General notes

My study suggests that the sex of the eels had no influence on the recovery of *A. crassus*. However, this result can be dependent on the strongly male- skewed sex ratio within the eels. Nevertheless, the lack of influence of eel sex on the infection parameters is on any account advantageous for the interpretation of the results, as the differences in immunological competence between eel sexes can be ignored (Lively et al., 2004).

The congruence of my data referring to recovery of the nematodes with the data from the literature confirms the appropriateness of experimental infections conducted in this trial. The percentage of the European worms recovered from the European eels ranged from 13.8% (10.4 SD) to 30.3% (17.7 SD) and is in agreement with previous findings of Haenen et al. (1996) (8% – 26%), De Charleroy et al. (1990) (maximum of 38.2%), Boon et al. (1990b) (maximum of 30%), Knopf (1999) (17.0 – 25.2%) and Knopf & Mahnke (2004) (33.2%).
In the Japanese eels infected with European larvae the recovery ranged between 5.3% (4.4 SD) and 44.5% (14.5 SD). Knopf & Mahnke (2004) reported of 13.8% European worms recovered from this eel species.
In the European eels infected with Taiwanese larvae 36.6% (11.9 SD) and 44.4% (13.1 SD) were recovered. In the Japanese eels infected with Taiwanese worms the recovery ranged from 25.9% (15.0 SD) to 4.0% (6.1 SD). To my knowledge no other references relating to two last host-parasite combinations are available.

On the one hand, the high heterogeneity of the recovery suggests that the individual L3 larvae may have a different migration time or migration route to the swim bladder of an eel. It is not clear whether the missing L3 missed their way or were killed by the unspecific immune response of the host. Heitlinger et al. (2009) showed that in the Japanese eel L3 larvae are already killed in the stomach and the intestine by enhanced infection intensities. However, I did not find any larvae in stomach or in the intestine of the European or the Japanese eels. On the other hand, the high overdispersion of the data (suggested by the high variances in relation to the mean values of recovery) is characteristic for macroparasite infections, where the smallest fraction of infected hosts harbours the highest number of the parasites. Similarly, aggregation of the parasites among their hosts was already found in field studies (Guyatt & Bundy, 1991; Münderle, 2005; Schabuss et al., 2005). Aggregated distributions of the parasites are common host-parasite patterns that allow for coexistence: the host profits from a lower density-dependent morbidity/mortality rate, while the assurance of the reproduction is advantageous for the parasite (Kennedy, 1994). The overdispersion of the

data is also mirrored in the non-random distribution of life history stages among the individual experimental eels.

The morphological investigations conducted in this study showed that all eel specimens were infected exclusively with *A. crassus*. A total of 1,279 and 585 adults sampled from the European eel and Japanese eel, respectively, were chosen for the morphological investigations. The substantially lower number of the worms from the Japanese eel was due to lower recovery of the nematode found in this eel species. However, both groups represent a statistically sufficient sample that should mirror the wide spectrum of morphological variability expressed by the worms.

The well documented sexual dimorphism of *A. crassus* (Moravec, 2006), with females being larger than the males, was incorporated within the framework of this study. The differences in dimensions of morphological traits between sexes occurred in both eel species on a comparable scale. The larger dimensions of females were accompanied with their faster growth suggesting more intensive blood intake by this sex. Münderle (2005) in his field study showed that the bodies of the females were longer and wider than those of the males by a factor 1.6 and 2.2 for the length and width, respectively. The inter-sex differences, in case of oesophagus und buccal capsule, were less pronounced at 1.2 and 1.1 for oesophagus und buccal capsule, respectively. The data presented by Münderle (2005) lay in the intervals that are comparable with those from this study. Interestingly, in contrast with the data of Münderle (2005), the females were characterized by a less compact oesophagus, which distinguishes this body part in the light of feeding behaviour. The differences in pharynx morphology linked to worm sex can be useful in studies dealing with the function of this feature.

A comparison of my own measurements of the body, oesophagus and buccal capsule of the European worms collected from the European eels with previous investigations of other authors revealed that the values lay in similar ranges, which confirms the correctness of the method used (Tables 27 - 29). However, direct comparisons of these values are not advisable due to the different fixation procedures used by different authors (frozen, fresh, cold formalin or ethanol). The European eels infected with the Taiwanese worms, as well as the Japanese eels infected with European worms, were not considered in this compilation, as no references to this host-parasite systems are available.

Table 27: The length and width of the body of *Anguillicola crassu* collected from the wild European eels in comparison with results of the current study.

Worm sex	Length (mm)	Width (mm)	Country	Sampling year	Reference
Female	2.0 – 54.0	0.2 – 4.6	Germany	2007 – 2008	This study
	16.2 – 36.7	1.36 – 5.0	Germany	1986 – 1987	Taraschewski et al., 1987
	3.0 – 56.0	0.3 – 4.9	Poland	2007 – 2008	This study
	8.0 – 35.0	0.4 – 4.1	Poland	2002 – 2005	Rolbiecki, 2008
	6.0 – 22.0		Netherlands	1987	Van Banning and Haenen, 1990
	1.8 – 60.5	0.2 – 6.7	Europe	2003 – 2004	Münderle, 2005
Male	3.0 – 35.0	0.2 – 2.5	Germany	2007 – 2008	This study
	5.8 – 23.1	0.3 – 1.8	Germany	1986 – 1987	Taraschewski et al., 1987
	3.0 – 40.0	0.2 – 2.3	Poland	2007 – 2008	This study
	6.0 – 26.0	0.51 – 2.3	Poland	2002 – 2005	Rolbiecki, 2008
	3.0 – 8.0		Netherlands	1987	Van Banning and Haenen, 1990
	1.3 – 42.9	0.1 – 2.6	Europe	2003 – 2004	Münderle, 2005

Table 28: The length and width of the oesophagus of *Anguillicola crassus* collected from the wild European eels in comparison with results of the current study.

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Female	540 – 1,110	250 – 470	Germany	2007 – 2008	This study
	775 – 1,060	258 – 381	Germany	1986 – 1987	Taraschewski et al., 1987
	590 – 1.170	170 – 440	Poland	2007 – 2008	This study
	604 – 895	181 – 301	Poland	2002 – 2005	Rolbiecki, 2008
	446 – 1,347	114 – 624	Europe	2003 – 2004	Münderle, 2005

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Male	470 – 980	110 – 470	Germany	2007 – 2008	This study
	571 – 816	135 – 258	Germany	1986 – 1987	Taraschewski et al., 1987
	510 – 1.000	150 – 410	Poland	2007 – 2008	This study
	528 – 755	91 – 287	Poland	2002 – 2005	Rolbiecki, 2008
	465 – 1,129	99 – 545	Europe	2003 – 2004	Münderle, 2005

Table 29: The length and width of the buccal capsule of *Anguillicola crassus* collected from the wild European eels in comparison with results of the current study.

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Female	15 – 25	40 – 68	Germany	2007 – 2008	This study
	24 – 27	54 – 63	Germany	1986 – 1987	Taraschewski et al., 1987
	16 – 27	42 – 70	Poland	2007 – 2008	This study
	18 – 29	44 – 66	Poland	2002 – 2005	Rolbiecki, 2008
	15 – 29	40 – 87	Europe	2003 – 2004	Münderle, 2005
Male	15 – 26	39 – 62	Germany	2007 – 2008	This study
	21 – 27	48 – 57	Germany	1986 – 1987	Taraschewski et al., 1987
	15 – 25	39 – 65	Poland	2007 – 2008	This study
	19 – 27	31 – 46	Poland	2002 – 2005	Rolbiecki, 2008
	13 – 28	39 – 81	Europe	2003 – 2004	Münderle, 2005

The measurements of the morphological traits of the Taiwanese population in the Japanese eel are in agreement with the data from the field presented by Taraschewski et al. (1987) and Münderle (2005). Conspicuous are the substantially higher ranges of the length and width of body, oesophagus and buccal capsule of the type specimen (Kuwahara et al., 1974). Again, this can be due to the hot formalin used as the fixation medium by the author, while ethanol

(current study and Münderle, (2005)) or cold formalin (Taraschewski et al., 1987) were used by other authors.

Table 30: The length and width of the body of *Anguillicola crassus* collected from the wild Japanese eels in comparison with results of the current study. N. a. – not available.

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Female	4.0 – 32.0	0.2 – 2.5	Taiwan	2007 – 2008	This study
	3.3 – 31.5	0.2 – 3.6	Taiwan	2003 – 2004	Münderle, 2005
	30.9 – 44.7	1.6 – 3.5	Japan	n. a.	Taraschewski et al., 1987
	47.1 – 71.5	3.0 – 5.6	Japan (type specimen)	n. a.	Kuwahara et al., 1974
Male	2.0 – 22.0	0.2 – 1.2	Taiwan	2007 – 2008	This study
	2.3 – 23.2	0.1 – 1.6	Taiwan	2003 – 2004	Münderle, 2005
	12.9 – 21.8	0.8 – 1.0	Japan	n. a.	Taraschewski et al., 1987
	20.5 – 55.9	0.9 – 2.8	Japan (type specimen)	n. a.	Kuwahara et al., 1974

Table 31: The length and width of the oesophagus of *Anguillicola crassus* collected from the wild Japanese eels in comparison with results of the current study. N. a. – not available.

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Female	560 – 1,060	100 – 350	Taiwan	2007 – 2008	This study
	540 – 1,040	119 – 347	Taiwan	2003 – 2004	Münderle, 2005
	911 – 1,090	231 – 272	Japan	n. a.	Taraschewski et al., 1987
	860 – 1,100	300 – 490	Japan (type specimen)	n. a.	Kuwahara et al., 1974
Male	470 – 890	120 – 270	Taiwan	2007 – 2008	This study
	361 – 871	69 – 248	Taiwan	2003 – 2004	Münderle, 2005
	775 – 843	207 – 218	Japan	n. a.	Taraschewski et al., 1987
	680 – 1,030	240 – 410	Japan (type specimen)	n. a.	Kuwahara et al., 1974

5. General notes

Table 32: The length and width of the buccal capsule of *Anguillicola crassus* collected from the wild Japanese eels in comparison with results of the current study. N. a. – not available.

Worm sex	Length (µm)	Width (µm)	Country	Sampling year	Reference
Female	15 – 26	41 – 62	Taiwan	2007 – 2008	This study
	14 – 24	39 – 69	Taiwan	2003 – 2004	Münderle, 2005
	27	60 – 63	Japan	n. a.	Taraschewski et al., 1987
	18 – 28	45 – 60	Japan (type specimen)	n. a.	Kuwahara et al., 1974
Male	12 – 24	38 – 66	Taiwan	2007 – 2008	This study
	13 – 22	38 – 61	Taiwan	2003 – 2004	Münderle, 2005
	21 – 27	48 – 60	Japan	n. a.	Taraschewski et al., 1987
	15 – 25	45 – 60	Japan (type specimen)	n. a.	Kuwahara et al., 1974

6 Conclusions and perspectives

The variation in recovery, developmental dynamics, reproductive output and morphology observed in *A. crassus* populations after their successful colonization of the European eel was induced by both genetic and plastic responses. Genetic divergence between both European parasite populations and the Taiwanese nematode strain in terms of infectivity and developmental dynamics is postulated. Moreover, the larger oesophagus dimensions expressed by the Polish parasite population, as well as the intraspecific variability in the morphology of the buccal capsule, are also genetically determined. In contrast, the reproductive output, as well as the size of body and (partially) the oesophagus, are plastic traits being differently expressed by individual genotypes when exposed to different environmental conditions. The ecological release constituted by the lack of immune response in the colonized host, lower water temperature in Europe or increased infrapopulation densities within an eel host are proposed as possible selective forces contributing to the observed genetic changes or plastically induced phenotypic variation among the populations of *A. crassus*.

Any kind of ecological release, such as the lack of predators in the colonized environment, is considered to be a relevant factor in the estimation of the colonization success (Lee, 2002). The Enemy Release Hypothesis (Dang et al., 2009) indicates that the invasiveness of introduced species is supported by the lack of specialized natural enemies in the recipient community (Facon et al., 2006). I propose an **Immune Release Hypothesis**, which states that a less effective immune response of a colonized host may elicit higher recovery (invasiveness, infectivity) of the source parasite population initiating the invasion event, faster development, higher growth rates, larger morphological dimensions, as well as higher reproductive output, of the established parasite population. The immunological release in the European eel seems to be a jumping off point for the successful invasion, allowing for high infection rates (recovery) and fast dissemination (larger body and oesophagus dimensions and higher reproductive output) of the colonizing parasite. After the establishment in the new host, local adaptation in terms of lowered recovery, faster development and changes in morphology of the oesophagus and buccal capsule has probably taken place. The genetic polymorphism of the parasite populations was less apparent in the Japanese eel, with no clear contribution to the fitness of the nematode being revealed.

For the future, it would be advisable to follow the European population in the Japanese eel for more than one generation in order to evaluate whether the genetic changes are reversible or develop in a new direction. In order to reveal whether temperature was the main selective factor, common garden experiments designed with different temperatures would be

recommended. Molecular investigation of European and Taiwanese populations based on a similar experimental design would substantially contribute to the understanding of the nature of the observed changes.

7 References

Addo-Bediako, A., Chown, S. L. & Gaston, K. J. (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16(3), 332–338.

Agrawal, A. A., Vala, F. & Sabelis, M. W. (2002). Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? *The American Naturalist* 159(5), 553–565.

Aieta, A. E. & Oliveira, K. (2009). Distribution, prevalence, and intensity of the swim bladder parasite *Anguillicola crassus* in New England and eastern Canada. *Diseases of aquatic organisms* 84(3), 229–235.

Allendorf, F. W. & Lundquist, L. L. (2003). Introduction: population biology, evolution, and control of invasive species. *Conservation Biology* 17(1), 24–30.

Anderson, R. M. (1991). Populations and infectious diseases: ecology or epidemiology? *The Journal of Animal Ecology* 60(1), 1–50.

Anderson, R. M. & May, R. M. (1978). Regulation and stability of host-parasite population interactions: I. Regulatory processes. *The journal of animal ecology* 47(1), 219–247.

Andreassen, J., Bennet-Jenkins, E. M. & Bryant, C. (1999). Immunology and biochemistry of Hymenolepis diminuta. *Advances in parasitology* 42, 223–275.

Aoyama, J. (2009). Life history and evolution of migration in catadromous eels (Genus *Anguilla*). *Aqua-BioScience Monographs* 2, 1–42.

Ashworth, S. T. & Kennedy, C. R. (1999). Density-dependent effects on *Anguillicola crassus* (Nematoda) within its European eel definitive host. *Parasitology* 118(3), 289–296.

Atkinson, D. & Sibly, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology & Evolution* 12(6), 235–239.

Audenaert, V., Huyse, T., Goemans, G., Belpaire, C. & Volckaert, F. A. M. (2003). Spatio-temporal dynamics of the parasitic nematode *Anguillicola crassus* in Flanders, Belgium. *Diseases of aquatic organisms* 56, 223–233.

Ayala, D., Caro-Riaño, H., Dujardin, J.-P., Rahola, N., Simard, F. & Fontenille, D. (2011). Chromosomal and environmental determinants of morphometric variation in natural populations of the malaria vector Anopheles funestus in Cameroon. *Infection, Genetics and Evolution* 11, 940-947.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A. G., Legout, H., Moreteau, B., Vergilino, R. & David, J. R. (2004). Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18(5), 700–706.

7. References

Bacevicus, E. (2004). The new data on ecology of alien fish species and fish metazoan parasites in the Curonian Lagoon and Lithuanian coastal zone. Baltic – the Sea of Aliens. Gdynia, Poland. *Book of Abstracts.* 17–18.

Baker, A. J. (1980). Morphometric differentiation in New Zealand populations of the house sparrow (*Passer domesticus*). *Evolution* 34(4), 638–653.

Van Banning, P. & Haenen, O. L. M. (1990). Effects of the swim bladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. *Pathology in marine science. Perkins, FO and TC Cheng. Academic Press. New York, USA*.

Van Banning, P., Heermans, W. & Van Willigen, J. A. (1985). *Anguillicola crassa*, een nieuwe aalparasiet in de Nederlandse wateren. *Visserij* 38, 237-240.

Barber, I. (2005). Parasites grow larger in faster growing fish hosts. *International Journal for Parasitology* 35(2), 137–143.

Barse, A. M. & Secor, D. H. (1999). An exotic nematode parasite of the American eel. *Fisheries* 24(2), 6–10.

Barse, A. M., McGuire, S. A., Vinores, M. A., Eierman, L. E. & Weeder, J. A. (2001). The swim bladder nematode *Anguillicola crassus* in American eels (*Anguilla rostrata*) from middle and upper regions of Chesapeake Bay. *The Journal of parasitology* 86(6), 1366–1370.

Baruš, V., Halacka, K. & Prokes, M. (1996). Presence and distribution of the nematode *Anguillicola crassus* in the European eel in the Morava river drainage basin, Czech Republic. *Zivocisna Vyroba* 41, 219-224.

Baruš, V., Prokes, M., Kracmar, S. & Tenora, F. (1998). Amino acid spectrum of uninfected and infected eels (*Anguilla anguilla*) by *Anguillicola crassus* (Nematoda). *Folia Zoologica* 47(3), 231-234.

Baruš, V., Tenora, F., Kracmar, S. & Dvoracek, J. (1999a). Contents of several inorganic substances in European eel infected and uninfected by *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms* 37(2), 135-137.

Baruš, V., Tenora, F., Kracmar, S., Prokes, M. & Dvoracek, J. (1999b). Microelement contents in males and females of *Anguillicola crassus* (Nematoda: Dracunculoidea). *Helminthologia (Bratislava)* 36(4), 283-285.

Békési, L., Hornok, S. & Székely, C. (1997). Attempts to analyse *Anguillicola crassus* infection and the humoral host response in eels (*Anguilla anguilla*) of Lake Balaton, Hungary. *Acta Veterinaria Hungarica* 45, 439–446.

Bell, G. (1982). The masterpiece of nature: the evolution and genetics of sexuality. *University of California Press, USA.*

7. References

Belpaire, C., De Charleroy, D., Grisez, L. & Ollevier, F. (1989a). Spreading mechanisms of the swim bladder parasite *Anguillicola crassus* in the European eel *Anguilla anguilla*, and its distribution in Belgium and Europe. *EIFAC–Working Group on Eel* 1–13.

Belpaire, C., De Charleroy, D., Thomas, K., Van Damme, P. & Ollevier, F. (1989b). Effects of eel restocking on the distribution of the swim bladder nematode *Anguillicola crassus* in Flanders, Belgium. *Journal of Applied Ichthyology* 5, 151–153.

Blanc, G., Bonneau, S., Biagianti, S. & Petter, A. J. (1992). Description of the larval stages of *Anguillicola crassus* (Nematoda, Dracunculoidea) using light and scanning electron microscopy. *Aquatic Living Resources* 5(4), 307–318.

Blondel, J., Thomas, D. W., Charmantier, A., Perret, P., Bourgault, P. & Lambrechts, M. M. (2006). A thirty-year study of phenotypic and genetic variation of blue tits in Mediterranean habitat mosaics. *Bioscience* 56(8), 661–673.

Blossey, B. & Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83(5), 887–889.

Boon, J. H., Lokin, C. J. A., Ceusters, R. & Ollevier, F. (1989). Some properties of the blood of European eel (*Anguilla anguilla*) and the possible relationship with *Anguillicola crassus* infestations. *Aquaculture* 76, 203-203.

Bonn, J. H., Augustijn, H., Cannaerts, V. M. H., Lokin, C. J. A., Michiels, M. A. M. & Ollevier, F. (1990). The suitability of experimental inoculations with infective larvae of *Anguillicola crassus* and their effects on the growth and mortality of the European eel, *Anguilla anguilla*. *Aquaculture* 87(2), 111-120.

Brämick, U., Fladung, E. & Doering-Arjes, P. (2008). Aalmamagementplan - Flussgebitsgemeinschaft Oder, Bericht. *Institut für Binnenfischerei eV Potsdam-Sacrow*.

Brown, S. P. & Grenfell, B. T. (2001). An unlikely partnership: parasites, concomitant immunity and host defence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268(1485), 2543-2549.

Bruňanská, M., Fagerholm, H. P. & Moravec, F. (2007). Structure of the pharynx in the adult nematode *Anguillicoloides crassus* (Nematoda: Rhabditida). *Journal of Parasitology* 93(5), 1017–1028.

Bruňanská, M., Fagerholm, H. P., Moravec, F. & Vasilková, Z. (2010). Ultrastructure of the buccal capsule in the adult female *Anguillicoloides crassus* (Nematoda: Anguillicolidae). *Helminthologia* 47(3), 170–178.

Buchmann, K. & Bjerregaard, J. (1990). Mebendazole treatment of pseudodactylogyrosis in an intensive eel-culture system. *Aquaculture* 86, 139–153.

7. References

Buchmann, K. & Bresciani, J. (1994). Ultrastructural evaluation of mebendazole action in *Pseudodactylogyrus bini* (Monogenea) a gill parasites from European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 19, 55-60.

Buchmann, K., Koie, M. & Prento, P. (1987a). The nutrition of the gill parasitic monogenean *Pseudodactylogyrus anguillae. Parasitology research* 73(6), 532–537.

Buchmann, K., Mellergaard, S. & Koie, M. (1987b). *Pseudodactylogyrus* infections in eel: a review. *Diseases of Aquatic Organisms* 3, 51–57.

Buchmann, K., Pedersen, L. O. & Glamann, J. (1991). Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Diseases of aquatic organisms* 12(1), 55–57.

Buchmann, K., Székely, C. & Bjerregaard, J. (1990). Trials with bunamidine, praziquantel and levamizole. *Bulletin European Association of Fish Pathologists* 10, 18–20.

Bush, A. O. & Lotz, J. M. (2000). The ecology of "crowding". *Journal of Parasitology* 86(2), 212-213.

Bush, A. O., Fernández, J. C., Esch, G. W., & Seed, J. R. (2001). Parasitism: the diversity and ecology of animal parasites. *Cambridge University Press, Cambridge, UK.*

Byrne, K. & Nichols, R. A. (1999). *Culex pipiens* in London underground tunnels: differentiation between surface and subterranean populations. *Heredity* 82(1), 7–15.

Cakić, D. P., Stojanovski, S., Kulišić, Z., Hristovski, N. & Lenhardt, M. (2002). Pojava *Anguillicola crassus*, nematoda: Dracunculoidea, kod jegulje iz Ohridskog jezera, Makedonija. *Acta veterinaria* 52, 163–168.

Campbell, T. S. & Echternacht, A. C. (2003). Introduced species as moving targets: changes in body sizes of introduced lizards following experimental introductions and historical invasions. *Biological Invasions* 5(3), 193–212.

Campbell, R. G., Wagner, M. M., Teegarden, G. J., Boudreau, C. A. & Durbin, E. G. (2001). Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Marine Ecology Progress Series* 221, 161–183.

Canestri-Trotti, G. (1987). Occurence of the Nematode *Anguillicola crassa* (Kuwahara, Niimi & Itagaki, 1974) in eels from the Po delta, Italy. *Bulletin-European Association of Fish Pathologists* 7(5), 109–111.

Carius, H. J., Little, T. J. & Ebert, D. (2001). Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. *Evolution* 55(6), 1136–1145.

Carlquist, S. (1974). Island Biology. *Columbia University Press, USA.*

7. References

Carreira, V., Soto, I., Hasson, E. & Fanara, J. (2006). Patterns of variation in wing morphology in the cactophilic *Drosophila buzzatii* and its sibling *D. koepferae*. *Journal of evolutionary biology* 19(4), 1275–1282.

Carroll, S. P. & Boyd, C. (1992). Host race radiation in the soapberry bug: natural history with the history. *Evolution* 46(4), 1052–1069.

Carroll, S. P., Dingle, H. & Klassen, S. P. (1997). Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. *Evolution* 51(4), 1182–1188.

Carroll, S. P., Klassen, S. P. & Dingle, H. (1998). Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. *Evolutionary Ecology* 12(8), 955–968.

Castonguay, M., Hodson, P. V., Moriarty, C., Drinkwater, K. F. & Jessop, B. M. (1994). Is there a role of ocean environment in American and European eel decline? *Fisheries Oceanography* 3(3), 197–203.

Chang, P. H., Pan, Y. H., Wu, C. M., Kuo, S. T. & Chung, H. Y. (2002). Isolation and molecular characterization of herpes-virus from cultured European eels *Anguilla anguilla* in Taiwan. *Diseases of aquatic organisms* 50(2), 111–118.

De Charleroy, D. & Thomas, K. (1989). The viability of the free living larvae of *Anguillicola* crassus. *Journal of Applied Ichthyology* 5, 154–156.

De Charleroy, D., Thomas, K. & Belpaire, C. (1987). Problems concerning the species determination, biology and diagnostical methods of *Anguillicola*, a swim bladder nematode in the European eel (*Anguilla anguilla* L.). *EIFAC Working Party on eel.*

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. & Ollevier, F. (1990). The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 8, 77-84.

Cheng, P. & Tzeng, W. (1996). Timing of metamorphosis and estuarine arrivalacross the dispersal range of the Japanese eel*Anguilla japonica*. *Marine Ecology Progress Series* 131, 87–96.

Clay, K. (2003). Parasites lost. *Nature* 421(6), 585–586.

Colautti, R. I. & MacIsaac, H. J. (2004). A neutral terminology to define'invasive'species. *Diversity and Distributions* 10(2), 135–141.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A. & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7(8), 721–733.

Combes, C. (2001). Parasitism: the ecology and evolution of intimate interactions. *University of Chicago Press, USA.*

Crean, S. R., Dick, J. T. A., Evans, D. W., Elwood, R. W. & Rosell, R. S. (2003). Anal redness in European eels as an indicator of infection by the swim bladder nematode, *Anguillicola crassus*. *Journal of Fish Biology* 62(2), 482–485.

7. References

Cruz, P., Silva, E., Grazina Freitas, M. S. & Carvalha-Varela, M. (1992). First report of *Anguillicola crassus* in the European eel in Portugal. *Bulletin-European Association of Fish Pathologists* 12, 154–154.

Dang, C., de Montaudouin, X., Bald, J., Jude, F., Raymond, N., Lanceleur, L., Paul-Pont, I. & Caill-Milly, N. (2009). Testing the enemy release hypothesis: trematode parasites in the non-indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia* 630(1), 139–148.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife–threats to biodiversity and human health. *Science* 287(5452), 443-449.

David, J., Gibert, P., Moreteau, B., Gilchrist, G. & Huey, R. (2003). The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura. Functional Ecology* 17(4), 425–430.

Dayan, T. & Simberloff, D. (1994). Character displacement, sexual dimprphism, and morphological variation among British and Irish mustelids. *Ecology* 75(4) 1063–1073.

Dekker, W. (2008). Coming to grips with the eel stock slip-sliding away. American Fisheries Society Symposium*, American Fisheries Society* 62, 335–355.

Dekker, W. & Van Willigen, J. (1989). Short note on the distribution and abundance of *Anguillicola* in the Netherlands. *Journal of Applied Ichthyology* 5(1), 46–47.

Denis, A., Gabrion, C. & Lambert, A. (1983). Two parasites of East-Asian origin reported in France: *Diplozoon nipponicum* Goto, 1891 (Monogenea) and *Botriocephalus acheilognathi* Yamaguti, 1934 (Cestoda) in *Cyprinus carpio* (Teleostei, Cyprinidae). *Bulletin Francais de Pisciculture* 55, 128-134.

Diegisser, T., Seitz, A. & Johannesen, J. (2007). Morphological adaptation in host races of *Tephritis conura. Entomologia experimentalis et applicata* 122(2), 155–164.

Dlugosch, K. M. & Parker, I. M. (2008). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters* 11(7), 701–709.

Dobson, C. & Owen, M. E. (1977). Influence of serial passage on the infectivity and immunogenicity of *Nematospiroides dubius* in mice. *International Journal for Parasitology* 7(6), 463–466.

Van Doorslaer, W., Stoks, R., Duvivier, C., Bednarska, A. & De Meester, L. (2009). Population dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution* 63(7), 1867–1878.

Dupont, F. & Petter, A. J. (1988). *Anguillicola*, a multispecific epizootic in Europe: appearance of *Anguillicola crassa* (Nematoda, Anguillicolidae) in the European eel *Anguilla anguilla* in Camargue, southern France. *Bull. Fr. Pêche Piscic* 308, 38–41.

7. References

Van Dyck, H., Matthysen, E. & Wiklund, C. (1998). Phenotypic variation in adult morphology and pupal colour within and among families of the speckled wood butterfly *Pararge aegeria*. *Ecological Entomology* 23(4), 465–472.

Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science* 265(5175), 1084-1086.

Edeline, E., Dufour, S. & Elie, P. (2005). Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Marine Ecology Progress Series* 304, 191–199.

Egusa, S. (1979). Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. *Rapports et proces-verbaux des Reunions* 174, 51-58.

El Hilali, M., Yahyaoui, A., Sadak, A., Maachi, M. & Taghy, Z. (1996). Premières données épidémiologiques sur l'anguillicolose au Maroc. *Bulletin Français de la Pêche et de la Pisciculture* 340, 57–60.

El-Rashidy, H. H. & Boxshall, G. A. (2009). Parasites gained: alien parasites switching to native hosts. *Journal of Parasitology* 95(6), 1326–1329.

Elton, C. S. (2000). The ecology of invasions by animals and plants. *University of Chicago Press, USA.*

Endler, J. A. (1986). Natural selection in the wild. *Princeton Univiversity Press. USA.*

Evans, D. W. & Matthews, M. A. (1999). *Anguillicola crassus* (Nematoda, Dracunculoidea); first documented record of this swim bladder parasite of eels in Ireland. *Journal of fish biology* 55(3), 665–668.

Ewald, P. W. (1995). The evolution of virulence: a unifying link between parasitology and ecology. *The Journal of parasitology* 81(5), 659–669.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A. & David, P. (2006). A general ecoevolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution* 21(3), 130–135.

Fazio, G. (2007). Epidémiologie et biologie du parasite invasif *Anguillicola crassus* (Nematoda) et son impact sur la physiologie de son hôte, l'anguille européenne, *Anguilla anguilla*. Doctoral thesis. Académie de Montpellier, Université de Perpignan, France.

Fazio, G., Moné, H., Lecomte-Finiger, R. & Sasal, P. (2008a). Differential gene expression analysis in European eels (*Anguilla anguilla*, L. 1758) naturally infected by macroparasites. *Journal of Parasitology* 94(3), 571-577.

Fazio, G., Sasal, P., Da Silva, C., Fumet, B., Boissier, J., Lecomte-Finiger, R. & Mone, H. (2008b). Regulation of *Anguillicola crassus* (Nematoda) infrapopulations in their definitive host, the European eel, *Anguilla anguilla*. *Parasitology* 135(14), 1707-1716.

7. References

Filchak, K. E., Roethele, J. B. & Feder, J. L. (2000). Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407(6805), 739–742.

Fox, C. W. & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology* 45(1), 341–369.

Fries, L. T., Williams, D. J. & Johnson, S. K. E. (1996). Occurrence of *Anguillicola crassus*, an exotic parasitic swim bladder nematode of eels, in the Southeastern United States. *Transactions of the American Fisheries Society* 125(5), 794–797.

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y. & Olivieri, I. (1996). Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proceedings: Biological Sciences* 263, 1003–1009.

Geets, A., Liewes, E. W. & Ollevier, F. (1992). Efficacy of some anthelmintics against the swim bladder nematode *Anguillicola crassus* of eel *Anguilla anguilla* under saltwater conditions. *Diseases of Aquatic Organisms* 13, 123–128.

Genc, E., Şahan, A., Altun, T., Cengizler, İ. & Nevsat, E. (2005). Occurrence of the swim bladder parasite *Anguillicola crassus* (Nematoda, Dracunculoidea) in European eels (*Anguilla anguilla*) in Ceyhan River, Turkey. *Turkish Journal of Veterinary and Animal Sciences* 29, 661–663.

Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21(3), 394–407.

Gilchrist, G. W., Jeffers, L. M., West, B., Folk, D. G., Suess, J. & Huey, R. B. (2008). Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura*. *Evolutionary Applications* 1(3), 513–523.

Van Ginneken, V., Ballieux, B., Willemze, R., Coldenhoff, K., Lentjes, E., Antonissen, E., Haenen, O. & van den Thillart, G. (2005). Hematology patterns of migrating European eels and the role of EVEX virus. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 140(1), 97–102.

Gollock, M. J., Kennedy, C. R., Quabius, E. S. & Brown, J. A. (2004). The effect of parasitism of European eels with the nematode, *Anguillicola crassus* on the impact of netting and aerial exposure. *Aquaculture* 233, 45-54.

Gordeeva, N. V. & Salmenkova, E. A. (2011). Experimental microevolution: transplantation of pink salmon into the European North. *Evolutionary Ecology* 25, 657-679.

Grapputo, A., Boman, S., Lindstroem, L., Lyytinen, A. & Mappes, J. (2005). The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology* 14, 4207–4219.

7. References

Greischar, M. A. & Koskella, B. (2007). A synthesis of experimental work on parasite local adaptation. *Ecology Letters* 10(5), 418–434.

Groman, J. D. & Pellmyr, O. (2000). Rapid evolution and specialization following host colonization in a yucca moth. *Journal of Evolutionary Biology* 13(2), 223–236.

Grosholz, E. (2002). Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology & Evolution* 17(1), 22–27.

Grosholz, E. D. & Ruiz, G. M. (2003). Biological invasions drive size increases in marine and estuarine invertebrates. *Ecology Letters* 6(8), 700–705.

Guyatt, H. & Bundy, D. (1991). Estimating prevalence of community morbidity due to intestinal helminths: prevalence of infection as an indicator of the prevalence of disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85(6), 778–782.

Haenen, O. L. M. & Van Banning, P. (1990). Detection of larvae of *Angullicola crassus* (an eel swim bladder nematode) in fresh water species. *Aquaculture* 87(2), 103-110.

Haenen, O. L. M., Van Banning, P. & Dekker, W. (1994a). Infection of eel *Anguilla anguilla* (L.) and smelt *Osmerus eperlanus* (L.) with *Anguillicola crassus* (Nematoda, Dracunculoidea) in the Netherlands from 1986 to 1992. *Aquaculture* 126, 219–229.

Haenen, O. L. M., Van Wijngaarden, T. A. M. & Borgsteede, F. H. M. (1994b). An improved method for the production of infective third-stage juveniles of *Anguillicola crassus*. *Aquaculture* 123, 163-165.

Haenen, O. L. M., Grisez, L., De Charleroy, D., Belpaire, C. & Ollevier, F. (1989). Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Diseases of aquatic organisms* 7(2), 97–101.

Haenen, O. L. M., Van Wijngaarden, T. A. M., Van der Heijden, M. H. T., Höglund, J., Cornelissen, J., Van Leengoed, L., Borgsteede, F. H. M. & Van Muiswinkel, W. B. (1996). Effects of experimental infections with different doses of *Anguillicola crassus* (Nematoda, Dracunculoidea) on European eel (*Anguilla anguilla*). *Aquaculture* 141, 41–57.

Hamilton, W. D., Axelrod, R. & Tanese, R. (1990). Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences of the United States of America* 87(9), 3566.

Hammerschmidt, K. & Kurtz, J. (2005). Evolutionary implications of the adaptation to different immune systems in a parasite with a complex life cycle. *Proceedings of the Royal Society B: Biological Sciences* 272, 2511-2518.

7. References

Han, Y. ., Chang, Y. ., Taraschewski, H., Chang, S. ., Chen, C. . & Tzeng, W. N. (2008). The swim bladder parasite *Anguillicola crassus* in native Japanese eels and exotic American eels in Taiwan. *Zoological Studies* 47(6), 667–675.

Harrison, L. (1915). Mallophaga from Apteryx, and their significance; with a note on the genus *Rallicola*. *Parasitology* 8(1), 88–100.

Van der Heijden, M. H. T., Helders, G. M., Booms, G. H. R., Huisman, E. A., Rombout, J. & Boon, J. H. (1996). Influence of flumequine and oxytetracycline on the resistance of the European eel against the parasitic swim bladder nematode *Anguillicola crassus*. *Veterinary immunology and immunopathology* 52, 127–134.

Heins, D. C., Baker, J. A. & Martin, H. C. (2002). The “crowding effect” in the cestode *Schistocephalus solidus*: density-dependent effects on plerocercoid size and infectivity. *Journal of Parasitology* 88(2), 302–307.

Heitlinger, E. G., Laetsch, D. R., Weclawski, U., Han, Y.-S. & Taraschewski, H. (2009). Massive encapsulation of larval *Anguillicoloides crassus i*n the intestinal wall of Japanese eels. *Parasites & Vectors* 2(48).

Hellstrom, A., Ljungberg, O. & Bornstein, S. (1988). *Anguillicola*, en ny ålparasit i Sverige. *Svensk Veterinartidning* 40 (4) 211–213.

Hendry, A. P., Hensleigh, J. E. & Reisenbichler, R. R. (1998). Incubation temperature, developmental biology, and the divergence of sockeye salmon (Oncorhynchus nerka) within Lake Washington. *Canadian Journal of Fisheries and Aquatic Sciences* 55(6), 1387–1394.

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C. & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* 290(5491), 516.

Hirose, H., Sekino, T. & Egusa, S. (1976). Notes on the egg deposition, larval migration and intermediate host of the nematode *Anguillicola crassa* parasitic in the swim bladder of eels. *Fish Pathology* 11, 27-31.

Hirose, H., Yabu, T., Hirono, I. & Aoki, T. (1998). The phylogeny of *Anguillicola crassus* and *A. globiceps* based on partial 18S ribosomal RNA sequences. *Journal of Fish Diseases* 21(4), 265-271.

Hoffman, G. (1990). *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. *Journal of Aquatic Animal Health* 2(1), 30–37.

Höglund, J. & Andersson, J. (1993). Prevalence and abundance of *Anguillicola crassus* in the European eel (*Anguilla anguilla*) at a thermal discharge site on the Swedish coast. *Journal of Applied Ichthyology* 9(2), 115–122.

7. References

Höglund, J. & Pilström, L. (1994). Purification of adult *Anguillicola crassus* whole-worm extract antigens for detection of specific antibodies in serum from the European eel (*Anguilla anguilla*) by ELISA. *Fish & Shellfish Immunology* 4(4), 311–319.

Höglund, J. & Pilström, L. (1995). Mechanical isolation and characterization of antigensfrom adult *Anguillicola crassus*. *Fish & Shellfish Immunology* 5(1), 51–60.

Höglund, J. & Thomas, K. (1992). The black goby *Gobius niger* as a potential paratenic host for the parasitic nematode *Anguillicola crassus* in a thermal effluent of the Baltic. *Diseases of aquatic organisms* 13(3), 175–180.

Höglund, J., Andersson, J. & Härdig, J. (1992). Haematological responses in the European eel, *Anguilla anguilla* L., to sublethal infestation by *Anguillicola crassus* in a thermal effluent of the Swedish Baltic. *Journal of Fish Diseases* 15(6), 507–514.

Holland, W. E., Smith, M. H., Gibbons, J. W. & Brown, D. H. (1974). Thermal tolerances of fish from a reservoir receiving heated effluent from a nuclear reactor. *Physiological zoology* 47(2), 110–118.

Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D. & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287(5451), 308-309.

Jakob, E., Neuhaus, H., Steinhagen, D., Luckhardt, B. & Hanel, R. (2009a). Monitoring of *Herpesvirus anguillae* (HVA) infections in European eel, *Anguilla anguilla* (L.), in northern Germany. *Journal of Fish Diseases* 32(6), 557–561.

Jakob, E., Walter, T. & Hanel, R. (2009b). A checklist of the protozoan and metazoan parasites of European eel (*Anguilla anguilla*): checklist of *Anguilla anguilla* parasites. *Journal of Applied Ichthyology* 25, 1-49.

James, A. C. & Partridge, L. (1995). Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *Journal of Evolutionary Biology* 8(3), 315–330.

James, A. C. & Partridge, L. (1998). Geographic variation in competitive ability in *Drosophila melanogaster*. *American Naturalist* 151, 530–537.

Johnsen, B. O. & Jenser, A. J. (1991). The *Gyrodactylus* story in Norway. *Aquaculture* 98, 289–302.

Johnson, S. K., Fries, L. T., Williams, J. & Huffman, D. G. (1995). Presence of the parasitic swim bladder nematode, *Anguillicola crassus*, in Texas aquaculture. *Texas aquaculture. World Aquaculture* 26(3), 35–36.

Johnston, R. F. & Selander, R. K. (1973). Evolution in the house sparrow. III. Variation in size and sexual dimorphism in Europe and North and South America. *American Naturalist* 107(955), 373–390.

7. References

Kaltz, O. & Shykoff, J. A. (1998). Local adaptation in host–parasite systems. *Heredity* 81(4), 361–370.

Kangur, A. (1994). Uus liik Vortsjaerve kalaparasiitide hulgas. *Eesti loodus* 6, 189.

Keane, R. M. & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17(4), 164–170.

Kennedy, C. R. (1994). The distribution and abundance of the nematode Anguillicola australiensis in eels Anguilla reinhardtii in Queensland, Australia. *Folia Parasitologica* 41(4), 279–286.

Kennedy, C. R. & Fitch, D. J. (1990). Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal of Fish Biology* 36(2), 117–131.

Kennedy, C. R., Di Cave, D., Berrilli, F. & Orecchia, P. (1996). Composition and structure of helminth communities in eels *Anguilla anguilla* from Italian coastal lagoons. *Journal of Helminthology* 71(1), 35-40.

Kennedy, C. R., Nie, P., Kaspers, J. & Paulisse, J. (2006). Are eels (*Anguilla anguilla* L.) planktonic feeders? Evidence from parasite communities. *Journal of Fish Biology* 41(4), 567–580.

Keymer, A. (1982). Density-dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology* 84(3), 573–587.

Kingsolver, J. G. & Pfennig, D. W. (2004). Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution* 58(7), 1608–1612.

Kirk, D. A. (2000). Effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae. *Diseases of aquatic organisms* 40, 211–218.

Kirk, R. S. (2003). The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* 10(6), 385–394.

Kirk, R. S., Lewis, J. W. & Kennedy, C. R. (2000). Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology* 120(3), 289–295.

Klerks, P. L. & Weis, J. S. (1987). Genetic adaptation to heavy metals in aquatic organisms: a review. *Environmental Pollution* 45(3), 173–205.

Knopf, K. (1999). Untersuchungen zur Immunantwort des Europäischen Aals (*Anguilla anguilla*) auf den Schwimmblasen-Nematoden *Anguillicola crassus*. Doctoral thesis, University of Karlsruhe, Germany.

Knopf, K. (2006). The swim bladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility

and immunity between a recently colonized host and the original host. *Journal of helminthology* 80(2), 129–136.

Knopf, K. & Lucius, R. (2008). Vaccination of eels (*Anguilla japonica* and *Anguilla anguilla*) against *Anguillicola crassus* with irradiated L3. *Parasitology* 135(5), 633–640.

Knopf, K. & Mahnke, M. (2004). Differences in susceptibility of the European eel (*Anguilla anguilla*) and the Japanese eel (*Anguilla japonica*) to the swim-bladder nematode *Anguillicola crassus*. *Parasitology* 129, 491-496.

Knopf, K., Würtz, J., Sures, B. & Taraschewski, H. (1998). Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Diseases of aquatic organisms* 33(2), 143.

Knopf, K., Naser, K., Van der Heijden, M. H. & Taraschewski, H. (2000). Humoral immune response of European eel *Anguilla anguilla* experimentally infected with *Anguillicola crassus*. *Diseases of aquatic organisms* 42(1), 61–69.

Knopf, K., Madriles H. A., Lucius, R., Bleiss, W. & Taraschewski, H. (2008). Migratory response of European eel (*Anguilla anguilla*) phagocytes to the eel swim bladder nematode *Anguillicola crassus*. *Parasitology Research* 102(6), 1311–1316.

Koie, M. (1988). Parasites in eels, *Anguilla anguilla* (L.), from eutrophic Lake Esrum (Denmark). *Acta Parasitologica Polonica* 33(2), 89–100.

Koie, M. (1991). Swim bladder nematodes (*Anguillicola* spp.) and gill monogeneans (*Pseudodactylogyrus* spp.) parasitic on the European eel (*Anguilla anguilla*). *ICES Journal of Marine Science* 47(3), 391-398.

Kolar, C. S. & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16(4), 199–204.

Kolbe, J. J., Larson, A., Losos, J. B. & De Queiroz, K. (2008). Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. *Biology letters* 4(4), 434–437.

Konecny, R. & Wais, A. (1993). Occurrence of *Anguillicola crassus* in eels of Lake Neusiedl, Austria. *Folia Parasitologica* 40, 327.

Koops, H. & Hartmann (1989). *Angulillicola*-infestations in Germany and in German eel imports. *Journal of Applied* Ichthyology 1, 41–45.

Kristmundsson, A. & Helgason, S. (2007). Parasite communities of ecls *Anguilla anguilla* in freshwater and marine habitats in Iceland in comparison with other parasite communities of eels in Europe. *Folia Parasitologica* 54(2), 141.

Kuwahara, A., Niimi, A. & Itagaki, H. (1974). Studies of a nematode parasitic in the air bladder of the eel: 1. Description of *Anguillicola crassus* n. sp. (Philometridea, Anguillicolidae). *Japanese Journal for Parasitology* 23(5), 275–279.

7. References

Lafferty, K. D. & Kuris, A. M. (1999). How environmental stress affects the impacts of parasites. *Limnology and Oceanography* 44(3), 925–931.

Laffont, R., Firmat, C., Alibert, P., David, B., Montuire, S. & Saucède, T. (2010). Biodiversity and evolution in the light of morphometrics: From patterns to processes. *Comptes Rendus Palevol* 10, 133-142.

Lambrinos, J. G. (2004). How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology* 85(8), 2061–2070.

Lätsch, D. (2010). The Anguillicolidae (Nematoda: Anguillicoloidea) Barcoding, population structures and phylogenetics. Diploma thesis, Karlsruhe Institute of Technology, Germany.

Lee, C. E. (1999). Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution* 53(5), 1423–1434.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology & Evolution* 17(8), 386–391.

Lefebvre, F. S. & Crivelli, A. J. (2004). Anguillicolosis: dynamics of the infection over two decades. *Diseases of aquatic organisms* 62, 227–232.

Lefebvre, F., Contournet, P. & Crivelli, A. J. (2002a). The health state of the eel swim bladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology* 124(4), 457-463.

Lefebvre, F., Contournet, P., Priour, F., Soulas, O. & Crivelli, A. J. (2002b). Spatial and temporal variation in *Anguillicola crassus* counts: results of a 4 year survey of eels in Mediterranean lagoons. *Diseases of aquatic organisms* 50(3), 181–188.

Liewes, E. W. & Schaminee-Main, S. (1987). Onderzoek aalparasiet vordert. *Aquacultuur* 2(4), 5–17.

Lively, C. M., Dybdahl, M. F., Jokela, J., Osnas, E. E. & Delph, L. F. (2004). Host sex and local adaptation by parasites in a snail-trematode interaction. *American Naturalist* 164(5), 6–18.

Lockwood, J. L., Cassey, P. & Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* 20(5), 223–228.

Lonsdale, W. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology* 80(5), 1522–1536.

Losos, J. B., Warheit, K. I. & Schoener, T. W. (1997). Adaptive differentiation following experimental island colonization in Anolis lizards. *Nature* 387(6628), 70–73.

Lowrie, F., Behnke, J. & Barnard, C. (2004). Density-dependent effects on the survival and growth of the rodent stomach worm *Protospirura muricola* in laboratory mice. *Journal of helminthology* 78(2), 121–128.

7. References

Lyndon, A. R. & Pieters, N. (2005). First record of the eel swim bladder parasite *Anguillicola crassus* (Nematoda) from Scotland. *Bulletin-European Association of Fish Pathologists* 25(2), 82.

Maamouri, F., Gargouri, L., Ould Daddah, M. & Bouix, G. (1999). Occurrence of *Anguillicola crassus* (Nematode, Anguillicolidae) in the Ichkeul Lake (Northern Tunisia). *Bulletin-European Association of Fish Pathologists* 19, 17–19.

MacDougall, A. S. & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86(1), 42–55.

Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M. & Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological applications* 10(3), 689–710.

Mackay, T. F. C. (2004). The genetic architecture of quantitative traits: lessons from *Drosophila*. *Current opinion in genetics & development* 14(3), 253–257.

Mackinnon, M., Gaffney, D. & Read, A. (2002). Virulence in rodent malaria: host genotype by parasite genotype interactions. *Infection, Genetics and Evolution* 1(4), 287–296.

MacNair, M. R. (1987). Heavy metal tolerance in plants: a model evolutionary system. *Trends in Ecology & Evolution* 2(12), 354–359.

Mahnke, M. (2002). *Vergleichende Untersuchungen zur Wirkung des Schwimmblasennematoden Anguillicola crassus beim Europäischen Aal (Anguilla anguilla) und Japanischen Aal (Anguilla japonica)*. Diploma thesis, Humbold Universität Berlin, Germany.

Medley, G. & Anderson, R. (1985). Density-dependent fecundity in *Schistosoma mansoni* infections in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 79(4), 532–534.

Mellergaard, S. (1990). Mebendazole treatment against *Pseudodactylogyrus* infections in eel (*Anguilla anguilla*). *Aquaculture* 91, 15–21.

Mideo, N. (2009). Parasite adaptations to within-host competition. *Trends in parasitology* 25(6), 261–268.

Miller, M., Mochioka, N., Otake, T. & Tsukamoto, K. (2002). Evidence of a spawning area of *Anguilla marmorata* in the western North Pacific. *Marine Biology* 140(4), 809–814.

Mladineo, I. & Maršić-Lučić, J. (2007). Host switch of *Lamellodiscus elegans* (Monogenea: Monopisthocotylea) and *Sparicotyle chrysophrii* (Monogenea: Polyopisthocotylea) between cage-reared sparids. *Veterinary research communications* 31(2), 153–160.

Mo, T. A. & Steien, S. H. (1994). First observation of the eel swim bladder nematode *Anguillicola crassus* in Norway. *Bulletin-European Association of Fish Pathologists* 14, 163–163.

7. References

Möller, H., Holst, S., Lüchtenberg, H. & Petersen, F. (1991). Infection of eel *Anguilla anguilla* from the River Elbe estuary with two nematodes, *Anguillicola crassus* and *Pseudodactylogyrus decipiens*. *Diseases of Aquatic Organisms* 11, 193–199.

Molnár, K. (1993). Effect of decreased oxygen content on eels (*Anguilla anguilla*) infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Acta Veterinaria Hungarica* 41(3-4), 349–360.

Molnár, K. (1994). Formation of parasitic nodules in the swim bladder and intestinal walls of the eel *Anguilla anguilla* due to infections with larval stages of *Anguillicola crassus*. *Diseases of aquatic organisms* 20(3), 163–170.

Molnár, K. & Székely, C. (1995). Parasitological survey of some important fish species of Lake Balaton. *Parasitologia Hungarica* 28, 63–82.

Molnár, K., Székely, C. & Baska, F. (1991). Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bulletin of the European Association of Fish Pathologists* 11(6), 211–212.

Molnár, K., Székely, C. & Perényi, M. (1994). Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection in eels of Lake Balaton, Hungary. *Folia Parasitologica* 41, 193–202.

Molnár, K., Szakolczai, J. & Vetési, F. (1995). Histological changes in the swim bladder wall of eels due to abnormal location of adults and second stage larvae of *Anguillicola crassus*. *Acta Veterinaria Hungarica* 43, 125–125.

Molnár, K., Baska, F., Csaba, G., Glávits, R. & Székely, C. (1993). Pathological and histopathological studies of the swim bladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Diseases of Aquatic Organisms* 15, 41–41.

Monath, T. P. (1994). Yellow fever and dengue–the interactions of virus, vector and host in the re-emergence of epidemic disease. *Proceedings of Seminars in virology* 5(2) 133–145.

Mooney, H. & Cleland, E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* 98(10), 5446-5451

Morand, S., Legendre, P., Gardner, S. L. & Hugot, J. P. (1996). Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecologia* 107(2), 274–282.

Moravec, F. (1992). Spreading of the nematode *Anguillicola crassus* (Dracunculoidea) among eel populations in Europe. *Folia Parasitologica (Ceske Budejovice)* 39(3), 247-248.

Moravec, F. (1994). Parasitic nematodes of freshwater fishes of Europe. *Kluwer Academic Publishers, Netherlands.*

7. References

Moravec, F. (1996). Aquatic invertebrates (snails) as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea) and the role of paratenic hosts in the life cycle of this parasite. *Diseases of Aquatic Organisms* 27, 237–239.

Moravec, F. (2006). Dracunculoid and anguillicoloid nematodes parasitic in vertebrates. *Academia, Czech Republic.*

Moravec, F. & Taraschewski, H. (1988). Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda: Anguillicolidae) of the swim bladder of eels, including descriptions of two new species, *A. novaezelandiae* sp. n. and *A. papernai* sp. n. *Folia parasitologica* 35(2), 125-146.

Moravec, F., Cave, D. D., Orecchia, P. & Paggi, L. (1994a). Experimental observations on the development of *Anguillicola crassus* (Nematoda: Dracunculoidea) in its definitive host, *Anguilla anguilla* (Pisces). *Folia Parasitologica* 41, 138-148.

Moravec, F., Di Cave, D., Orecchia, P. & Paggi, L. (1994b). Present occurrence of *Anguillicola novaezelandiae* (Nematoda: Dracunculoidea) in Europe and its development in the intermediate host. *Folia Parasitologica* 41, 203–203.

Morgan, J. A. T., Dejong, R. J., Adeoye, G. O., Ansa, E., Barbosa, C. S., Bremond, P., Cesari, I. M., Charbonnel, N., Correa, L. R., Coulibaly, G. & others (2005). Origin and diversification of the human parasite *Schistosoma mansoni*. *Molecular Ecology* 14(12), 3889–3902.

Morozinska-Gogol, J. (2005). Occurance of Asiatic nematode *Anguillicola crassus* in European eel from Łebsko Lagoon (Central coast, Poland). *Oceanological and Hydrobiological Studies* 34, 113-119.

Moser, M. L., Patrick, W. S. & Crutchfield Jr, J. U. (2000). Incidence of *Anguillicola crassus* infection of American eels in North Carolina waters. *Final Report to North Carolina Fisheries Research Grant Program, Project* 42.

Münderle, M. (2005). Ökologische, morphologische und genetische Untersuchungen an Populationen des invasiven Schwimmblasen-Nematoden *Anguillicola crassus* aus Europa und Taiwan. Doctoral thesis. University of Karlsruhe, Germany.

Münderle, M., Taraschewski, H., Klar, B., Chang, C. W., Shiao, J. C., Shen, K. N., He, J. T., Lin, S. H. & Tzeng, W. N. (2006). Occurrence of *Anguillicola crassus* (Nematoda: Dracunculoidea) in Japanese eels *Anguilla japonica* from a river and an aquaculture unit in SW Taiwan. *Diseases of aquatic organisms* 71(2), 101–108.

Nabil, L., Abderrafik, M. & Boudjema, S. (2009). Biodiversité des Parasites chez *Anguilla anguilla* Linnaeus, 1758 dans le Parc National d'El Kala – Algérie. *European Journal of Scientific Research* 25(2), 300–309.

7. References

Nadler, S. A., Carreno, R. A., Mejía-Madrid, H., Ullberg, J., Pagan, C., Houston, R. & Hugot, J. P. (2007). Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* 134(10), 1421–1442.

Nagasawa, K., Kim, Y. G. & Hirose, H. (1994). *Anguillicola crassus* and *A. globiceps* (Nematoda: Dracunculoidea). *Folia Parasitologica* 41, 127–137.

Neumann, W. (1985). Schwimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* 11, 322.

Neves, R. H., Costa-Silva, M., Martinez, E. M., Branquinho, T. B., Oliveira, R. M. F., Lenzi, H. L., Gomes, D. C. & Machado-Silva, J. R. (2004). Phenotypic plasticity in adult worms of *Schistosoma mansoni* (Trematoda: Schistosomatidae) evidenced by brightfield and confocal laser scanning microscopies. *Memórias do Instituto Oswaldo Cruz* 99(2), 131–136.

Neveu, A. (1981). Variations saisonnières et journalières de l"alimentation de l"anguille (*Anguilla anguilla* L.) dans des conditions naturelles. *Acta Oecologica, Oecologia Applicata* 2, 99–116.

Nielsen, M. E. (1997). Infection status of the swim bladder worm, *Anguillicola crassus* in silver stage European eel, *Anguilla anguilla*, from three different habitats in Danish waters. *Journal of Applied Ichthyology* 13(4), 195-196.

Nielsen, M. E. (1999). An enhanced humoral immune response against the swim bladder nematode, *Anguillicola crassus*, in the Japanese eel, *Anguilla japonica*, compared with the European eel, *A. anguilla*. *Journal of helminthology* 73(3), 227–232.

Nielsen, M. E. & Buchmann, K. (1997). Glutathione-s-transferase is an important antigen in the eel nematode *Anguillicola crassus*. *Journal of helminthology* 71(4), 319–324.

Nielsen, M. E. & Esteve-Gassent, M. D. (2006). The eel immune system: present knowledge and the need for research. *Journal of Fish Diseases* 29(2), 65–78.

Niewiarowski, P. H. & Roosenburg, W. (1993). Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus. Ecology* 74(7) 1992–2002.

Nimeth, K., Zwerger, P., Würtz, J., Salvenmoser, W. & Pelster, B. (2000). Infection of the glass-eel swim bladder with the nematode *Anguillicola crassus*. *Parasitology* 121(1), 75–83.

Nuismer, S. L. & Gandon, S. (2008). Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. *American Naturalist* 171 (5), 658–668.

Orecka, T., Pilecka-Rapacz, M. & Rzad, I. (1995). Pasożyty – azjatyckie nicienie – przyczyna wyniszczającego schorzenia węgorzy. *Przegląd Wędkarski* 2, 50-55.

7. References

Paggi, L., Orecchia, P., Minervini, R. & Mattiucci, S. (1982). Sulla comparsa di *Anguillicola australiensis* Johnston e Mason, 1940 (Dracunculoidea: Anguillicolidae) in *Anguilla anguilla* del lago di Bracciano. *Parassitologia* 24, 139–144.

Palikova, M. & Navratil, S. (2001). Occurrence of *Anguillicola crassus* in the Water Reservoir Korycany (Czech Republic) and its influence on the health condition and haematological indices of eels. *Acta Veterinaria Brno* 70(4), 443-449.

Palstra, A. P., Heppener, D. F. M., van Ginneken, V. J. T., Székely, C. & van den Thillart, G. (2007). Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. *Journal of Experimental Marine Biology and Ecology* 352(1), 244–256.

Pappers, S. M., van der Velde, G., Ouborg, N. J. & van Groenendael, J. M. (2002). Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphaeae. Evolution* 1610–1621.

Paterson, S. & Barber, R. (2007). Experimental evolution of parasite life-history traits in *Strongyloides ratti* (Nematoda). *Proceedings of the Royal Society B: Biological Sciences* 274(1617), 1467-1474.

Paterson, S. & Viney, M. (2002). Host immune responses are necessary for density dependence in nematode infections. *Parasitology* 125(3), 283–292.

Pazooki, J. & Szekely, C. S. (1994). Survey of the paratenic hosts of *Anguillicola crassus* in Lake Velence, Hungary. *Acta Veterinaria Hungarica* 42, 87–87.

Peters, G. & Hartmann, F. (1986). *Anguillicola,* a parasitic nematode of the swim bladder spreading among eel populations in Europe. *Diseases of Aquatic Organisms* 1(3), 229–230.

Petter, J., Cassone, J. & Le Belle, N. (1990). Observations sur la biologie des premiers stades larvaires d'*Anguillicola crassus*, nématode parasite d'anguille. *Annales de parasitologie humaine et comparée* 65(1), 28–31.

Phillips, B. L. & Shine, R. (2006). An invasive species induces rapid adaptive change in a native predator: cane toads and black snakes in Australia. *Proceedings of the Royal Society B: Biological Sciences* 273(1593), 1545.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution* 20(9), 481–486.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50(1), 53–65.

7. References

Pimentel, D., Zuniga, R. & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52(3), 273–288.

Polzer, M. & Taraschewski, H. (1993). Identification and characterization of the proteolytic enzymes in the developmental stages of the eel-pathogenic nematode *Anguillicola crassus*. *Parasitology Research* 79(1), 24–27.

Price, P. W. (1977). General concepts on the evolutionary biology of parasites. *Evolution* 31(2), 405–420.

Puqin, W. & Yuru, Z. (1980). Observations on the life history of *Angllillicola globiceps* (Nematoda: Anguillicolidae). *Acta Zoologica Sinica* 26, 243-249.

Quinn, T. P., Kinnison, M. T. & Unwin, M. J. (2001). Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. *Genetica* 112(1), 493–513.

R Development Core Team (2009). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahhou, I., Morand, S., Lecomte-Finiger, R. & Sasal, P. (2005). Biogeographical relationships of the eel parasite *Anguillicola crassus* revealed by random amplified polymorphic DNA markers (RAPDs). *Bulletin Francais de la Peche et de la Pisciculture* 378, 87–98.

Reeve, M., Fowler, K. & Partridge, L. (2000). Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *Journal of Evolutionary Biology* 13(5), 836–844.

Reimer, L. (2002). Veränderungen im Parasitenbefall der Aale der südlichen Ostseeküste in den 90er Jahren. *Fischer & Teichwirt* 10, 384-385.

Reimer, L. W., Hildebrand, A., Scharberth, D. & Walter, U. (1994). *Anguillicola crassus* in the Baltic Sea: field data supporting transmission in brackish waters. *Diseases of Aquatic Organisms* 18(1), 77–79.

Reznick, D. N. & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112(1), 183–198.

Reznick, D. A., Bryga, H. & Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature* 346(6282), 357–359.

Roberts, L. S. (2000). The crowding effect revisited. *Journal of Parasitology* 86(2), 209–211.

Rohde, K. (1991). Intra- and interspecific interactions in low density populations in resource rich habitats. *Oikos* 60(1), 91–104.

7. References

Rolbiecki, L. (2002). On the role of paratenic hosts in the life cycle of the nematode *Anguillicola crassus* in the Vistula Lagoon, Poland. *Acta Ichtyologica et Piscatoria* 32(2), 109-116.

Rolbiecki, L. (2008). New data on the biology of the introduced exotic nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 in the eel *Anguilla anguilla* in Lake Wdzydze (Polish waters). *Oceanological & Hydrobiological Studies* 37(3), 37-48.

Rolbecki, L. & Rokicki, J. (2005). *Anguillicola crassus* - an alien nematode species from the swim bladders of eel (*Anguilla anguilla*) in the Polish zone of the southern Baltic and in the waters of northern Poland. *International Journal of Oceanography and Hydrobiology* 35(1), 121-136.

Rolbiecki, L., Rokicki, J. & Wojtkiewicz, D. (2000). The first record of the nematode *Anguillicola crassus* (Nematoda: Dracunculoidea) in eel of the Gulf of Gdansk (Poland). *Oceanological Studies* 29(2), 75-81.

Roman, J. & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* 22(9), 454–464.

Rosenheim, J. A., Johnson, M. W., Mau, R. F. L., Welter, S. C. & Tabashnik, B. E. (1996). Biochemical Per adaptations, Founder Events, and the Evolution of Resistance in Arthropods. *Journal of economic entomology* 89(2), 263–273.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C. & others (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* 32, 305–332.

Saroglia, M. G., Fantin, P. & Arlati, G. (1985). Eel production in Italy - problems and perspectives. *Report of the European Inland Fishery Advisory Commission (FAO), Working Party on Eel, Perpignan*, *France.*

Sarti, M., Giorgetti, G., Brisinello, W. & Vanelli, M. (1985). A new problem for intensive eel rearing in Italy: *Anguillicola australiensis. Proceedings of Proc. EAFP Congress, Montpellier*, *France.*

Sasal, P., Taraschewski, H., Valade, P., Grondin, H., Wielgoss, S. & Moravec, F. (2008). Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. *Parasitology research* 102(6), 1343–1350.

Sasal, P., Trouvé, S., Müller-Graf, C. & Morand, S. (1999). Specificity and host predictability: a comparative analysis among monogenean parasites of fish. *Journal of Animal Ecology* 68(3), 437–444.

Schabuss, M., Kennedy, C. R., Konecny, R., Grillitsch, B., Reckendorfer, W., Schiemer, F. & Herzig, A. (2005). Dynamics and predicted decline of *Anguillicola crassus* infection in

European eels, *Anguilla anguilla in Neusiedler See, Austria. Journal of Helminthology* 79, 159–167.

Schmahl, G. (1991). The chemotherapy of monogeneans which parasitize fish: a review. *Folia Parasitologica* 38, 97–106.

Scholz, T. (1999). Parasites in cultured and feral fish. *Veterinary Parasitology* 84, 317–335.

Seeley, R. H. (1986). Intense natural selection caused a rapid morphological transition in a living marine snail. *Proceedings of the National Academy of Sciences* 83(18), 6897-6901.

Sexton, J. P., McKay, J. K. & Sala, A. (2002). Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications* 12(6), 1652–1660.

Sicard, D., Pennings, P. S., Grandclément, C., Acosta, J., Kaltz, O. & Shykoff, J. A. (2007). Specialization and local adaptation of a fungal parasite on two host plant species as revealed by two fitness traits. *Evolution* 61(1), 27–41.

Singer, M. C., Thomas, C. D. & Parmesan, C. (1993). Rapid human-induced evolution of insect–host associations. *Nature* 366(6456), 681–683.

Sire, C., Rognon, A. & Théron, A. (1998). Failure of *Schistosoma mansoni* to reinfect *Biomphalaria glabrata* snails: acquired humoral resistance or intraspecific larval antagonism? *Parasitology* 117(2), 117–122.

Sjöberg, N. B., Petersson, E., Wickström, H. & Hansson, S. (2009). Effects of the swim bladder parasite *Anguillicola crassus* on the migration of European silver eels *Anguilla anguilla* in the Baltic Sea. *Journal of Fish Biology* 74(9), 2158–2170.

Smith, L. D. (2004). Biogeographic differences in claw size and performance in an introduced crab predator *Carcinus maenas*. *Marine Ecology Progress Series* 276, 209–222.

Smith, T. B., Freed, L. A., Lepson, J. K. & Carothers, J. H. (1995). Evolutionary consequences of extinctions in populations of a Hawaiian honeycreeper. *Conservation Biology* 9(1), 107–113.

Sorci, G., Skarstein, F., Morand, S. & Hugot, J. P. (2003). Correlated evolution between host immunity and parasite life histories in primates and oxyurid parasites. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1532), 2481.

Strayer, D. L., Eviner, V. T., Jeschke, J. M. & Pace, M. L. (2006). Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution* 21(11), 645–651.

Suarez, A. V. & Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Molecular Ecology* 17(1), 351–360.

Sures, B. & Knopf, K. (2004a). Individual and combined effects of cadmium and 3, 3′, 4, 4′, 5-pentachlorobiphenyl (PCB 126) on the humoral immune response in European eel

(*Anguilla anguilla*) experimentally infected with larvae of *Anguillicola crassus* (Nematoda). *Parasitology* 128(4), 445–454.

Sures, B. & Knopf, K. (2004b). Parasites as a threat to freshwater eels? *Science* 304(5668), 209-211.

Sures, B., Knopf, K. & Kloas, W. (2001). Induction of stress by the swim bladder nematode *Anguillicola crassus* in European eels, *Anguilla anguilla*, after repeated experimental infection. *Parasitology* 123(2), 179–184.

Sures, B., Knopf, K. & Taraschewski, H. (1999a). Development of *Anguillicola crassus* (Dracunculoidea, Anguillicolidae) in experimentally infected *Balearic congers Ariosoma balearicum* (Anguilloidea, Congridae). *Diseases of Aquatic Organisms* 39(1), 75-78.

Sures, B., Lutz, I. & Kloas, W. (2005). Effects of infection with *Anguillicola crassus* and simultaneous exposure with Cd and 3, 3′, 4, 4′, 5-pentachlorobiphenyl (PCB 126) on the levels of cortisol and glucose in European eel (*Anguilla anguilla*). *Parasitology* 132(2), 281–288.

Sures, B., Knopf, K., Würtz, J. & Hirt, J. (1999b). Richness and diversity of parasite communities in European eels *Anguilla anguilla* of the River Rhine, Germany, with special reference to helminth parasites. *Parasitology* 119(03), 323–330.

Szekely, C. (1994). Paratenic hosts for the parasitic nematode *Anguillicola crassus* in Lake Balaton, Hungary. *Diseases of Aquatic Organisms* 18(1), 11-20.

Szekely, C. (1996). Host reaction in paratenic fish hosts against 3rd stage larvae of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 26, 173–180.

Székely, C., Lang, M. & Csaba, G. (1991). First occurrence of *Anguillicola crassus* in Hungary. *Bulletin of the European Association of Fish Pathologists* 11(4), 162-163.

Taraschewski, H. (2006). Hosts and parasites as aliens. *Journal of helminthology* 80(2), 99–128.

Taraschewski, H., Moravec, F., Lamah, T. & Anders, K. (1987). Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Diseases of aquatic organisms* 3(3), 167–176.

Teng, H. Y., Lin, Y. S. & Tzeng, C. S. (2009). A new *Anguilla* species and a reanalysis of the phylogeny of freshwater eels. *Zoological Studies* 48(6), 808–822.

Ternengo, S., Levron, C., Desideri, F. & Marchand, B. (2005). Parasite communities in European eels *Anguilla anguilla* (Pisces, Teleostei) from a Corsican coastal pond. *Vie et milieu* 55(1), 1–6.

7. References

Tesch, F. W. (2003). The eel. 3rd ed. *Blackwell Science and the Fisheries Society of the British Isles, UK.*

Thébaud, C. & Simberloff, D. (2001). Are plants really larger in their introduced ranges? *The American Naturalist* 157(2), 231–236.

Thielen, F. (2005). Der Einfluss einwandernder Amphipodenarten auf die Parasitozönose des Europäischen Aals (*Anguilla anguilla*). Doctoral thesis, University of Karlsruhe, Germany.

Thomas, K. & Ollevier, F. (1992a). Paratenic hosts of the swim bladder nematode *Anguillicola crassus*. *Diseases of Aquatic Organisms* 13, 165–174.

Thomas, K. & Ollevier, F. (1992b). Population biology of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Diseases of Aquatic Organisms* 14(3), 163-170.

Thomas, K. & Ollevier, F. (1993). Hatching, survival, activity and penetration efficiency of second-stage larvae of *Anguillicola crassus* (Nematoda). *Parasitology* 107(2), 211–217.

Thulin, C. G., Simberloff, D., Barun, A., Mccracken, G., Pascal, M. & Islam, M. A. (2006). Genetic divergence in the small Indian mongoose (*Herpestes auropunctatus*), a widely distributed invasive species. *Molecular Ecology* 15(13), 3947–3956.

Trouvé, S., Sasal, P., Jourdane, J., Renaud, F. & Morand, S. (1998). The evolution of life-history traits in parasitic and free-living platyhelminthes: a new perspective. *Oecologia* 115(3), 370–378.

Tschirren, B., Fitze, P. S. & Richner, H. (2003). Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings. *Journal of Animal Ecology* 72(5), 839–845.

Tsutsui, N. D. & Suarez, A. V. (2003). The colony structure and population biology of invasive ants. *Conservation biology* 17(1), 48–58.

Ushikoshi, R., Inui, T., Mano, N. & Hirose, H. (1999). A method for specific antibody detection from Japanese eel by indirect ELISA using cuticular antigen of *Anguillicola crassus*. *Fish Pathology* 34, 81–82.

Via, S., Bouck, A. C. & Skillman, S. (2000). Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution* 54(5), 1626–1637.

Vismanis, K., Kirjusina, M. & Rodzins, R. (1999). An eel nematode *Anguillicola crassus* Kuwahara, Nimii et Itagaki, 1974 (Nematoda: Dracunculoidea) in Latvia. *Proceedings of the Fifth International Symposium on Fish Parasites, Czech Republic.*

7. References

Waller, P. J. & Buchmann, K. (2001). Anthelmintic resistance and parasite control in commercial eel farms: consequences for producers. *The Veterinary Record* 148(25), 783-784.

Wallet, M. & Lambert, A. (1984). Characterization of the cercaria of *Bucephalus polymorphus* Baer, 1827 (Trematoda, Bucephalidae): chetotaxy and excretory system. *Annales de parasitologie humaine et comparée* 59(6), 583-588.

Watanabe, S. (2001). Taxonomy of the freshwater eels, genus *Anguilla. Aquabiology* 23(2) 115-122.

Wescott, R. B. & Todd, A. C. (1966). Adaptation of *Nippostrongylus brasiliensis* to the mouse. *The Journal of Parasitology* 52(2), 233–236.

West-Eberhard, M. J. (2003). Developmental plasticity and evolution. *Oxford University Press, USA.*

West-Eberhard, M. J. (2005). Phenotypic accommodation: adaptive innovation due to developmental plasticity. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 304(6), 610–618.

Wielgoss, S., Taraschewski, H., Meyer, A. & Wirth, T. (2008). Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. *Molecular ecology* 17(15), 3478–3495.

Wierzbicka, J. (1994). Revision of the *Sphaerospora thélohan*, 1892 (Myxosporea) protozoans parasitising the eel, *Anguilla anguilla* (L). *Acta Ichtyologica et Piscatoria* 24(2), 21-24.

Wijová, M., Moravec, F., Horák, A. & Lukes, J. (2006). Evolutionary relationships of Spirurina (Nematoda: Chromadorea: Rhabditida) with special emphasis on dracunculoid nematodes inferred from SSU rRNA gene sequences. *International journal for parasitology* 36(9), 1067–1075.

Wild, G., Gardner, A. & West, S. A. (2009). Adaptation and the evolution of parasite virulence in a connected world. *Nature* 459(7249), 983–986.

Williams, C. & Moore, R. J. (1989a). Phenotypic adaptation and natural selection in the wild rabbit, *Oryctolagus cuniculus*, in Australia. *The Journal of Animal Ecology* 58(2) 495–507.

Williams, C. K. & Moore, R. J. (1989b). Genetic divergence in fecundity of Australian wild rabbits *Oryctolagus cuniculus. The Journal of Animal Ecology* 58(1) 249–259.

Williamson, M. H. & Fitter, A. (1996). The characters of successful invaders. *Biological conservation* 78, 163–170.

7. References

Wlasow, T., Gomulka, P., Martyniak, A., Boron, S., Hliwa, P., Terlecki, J. & Szymanska, U. (1998). *Anguillicola crassus* larvae in cormorant's prey fish in Vistula Lagoon, Poland. *Bulletin Francais de la Peche et de la Pisciculture* 349, 223-227.

Własow, T., Gomułka, P., Martyniak, A. & Hliwa, P. (1998). Ruffe *Gymnocephalus cernua* L. the main paratenic host of the nematode *Anguillicola crassus* in Vistula Lagoon waters. *Wiadomosci Parazytologiczne* 44, 365.

Wolfe, L. M. (2002). Why alien invaders succeed: support for the escape-from-enemy hypothesis. *The American Naturalist* 160(6), 705–711.

Würtz J, D. A. (1998). Distribution and prevalence of *Anguillicola crassus* (Nematoda) in eels *Anguilla anguilla* of the rivers Rhine and Naab, Germany. *Diseases of aquatic organisms* 32, 137–143.

Würtz, J. & Taraschewski, H. (2000). Histopathological changes in the swim bladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. *Diseases of aquatic organisms* 39(2), 121–134.

Würtz, J., Taraschewski, H. & Pelster, B. (1996). Changes in gas composition in the swim bladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology* 112(2), 233-238.

Zaostrovceva, S. (1993). Ekologo-faunisticheskij obzor gelmintofauny ryb Kaliningradskogo Zaliva. *Nauchno-tekhnicheskaja Konferencija Professorsko-Prepodavatel'skogo Sostava, Aspirantov i Sotrudnikov Kaliningradskogo Tekhnicheskogo Instituta Rybnoj Promyshlennosti i Khozjajstva, Tezisy dokladov* 18.

Zholdasova, I. (1997). Sturgeons and the Aral Sea ecological catastrophe. *Environmental biology of fishes* 48(1), 373–380.

Internet references:

Fishbase, available:

http://www.fishbase.org/summary/Anguilla-japonica.html

http://www.fishbase.org/summary/speciessummary.php?id=35

The IUCN Red List of Threatened Species, available:

http://www.iucnredlist.org/apps/redlist/details/60344/0

Die Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW), available:

7. References

http://www.lubw.baden–wuerttemberg.de/servlet/is/72252/

DAISIE, Delivering Alien Invasive Species Inventories for Europe, available:

http://www.europe-aliens.org/speciesTheWorst.do

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