Metazoan parasite communities in Alosa alosa (Linnaeus, 1758) and Alosa fallax (Lac,pSde, 1803) (Clupeidae) from North-East Atlantic coastal waters and connected rivers

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Abstract

Metazoan parasites were studied in 96 *Alosa alosa* and 78 *Alosa fallax* from North-East Atlantic coastal waters and connected rivers (among them three sympatric sites) in order to increase knowledge on these anadromous endangered fish and measure the parasitic impact on host condition.

All shads were infected by one to six metazoan parasite taxa among the 12 identified in the whole sampling, with a mean abundance of parasites higher for *A. alosa* (167 ± 10) than for *A. fallax* (112 ± 11). Helminths, mostly trophically-transmitted, were the best represented (eight taxa, prevalence up to 99%) in contrast with crustaceans and Petromyzontidae that rarely occurred (four taxa, prevalence < 6%). Despite some quantitative differences, metazoan parasite communities of *A. alosa* and *A. fallax* remained stable in composition whatever host developmental stage, sex, sample site and salinity. Among the nine parasite taxa harbored by each *Alosa* species, six were shared with some differences in distribution patterns including in sympatric conditions, suggesting increasing dissimilarities between *A. alosa* and *A. fallax* with the age. Information on feeding ecology provided by trophically-transmitted helminths confirmed euryphagous opportunistic diet of immatures and adults of both shad species, and assessed feeding of adults during spawning migrations. Our study also revealed the significant negative impact of *Hemiurus appendiculatus* on *A. alosa* and *Pronoprymna ventricosa* on *A. fallax*.

Because helminth parasites are omnipresent in the shads and decrease their fitness, parasitological data must be included in further investigations and management programs on *A. alosa* and *A. fallax*.

Key-words

Alosa spp.; metazoan parasites; host developmental stage; marine vs freshwater phases; fitness loss.

Introduction

Although cryptic, parasites are ubiquitous members of ecological communities, representing high biomass, and are recognized as key players in broader interactions and ecosystem dynamics, such as food web structure and energy flow (e.g. Price et al. 1986; Marcogliese 2004; Kuris et al. 2008; Johnson et al. 2010; Hatcher et al. 2012; Lambden and Johnson 2013; Sekalovic et al. 2014). Parasites increase vulnerability of their host and decrease their fitness; the host mortality risk of infected individuals being almost thrice higher compared to hosts uninfected or with reduced parasite burdens (Combes 1995; Thomas et al. 2007; Robar et al. 2010; McElroy and De Buron 2014 for reviews). Furthermore, parasites such as helminths are increasingly used as biological tags to provide information on host populations (e.g. feeding habits, habitat use, stock discrimination, and migration) and on free-living biodiversity and changes in ecosystem structure and functioning (MacKenzie 2002; Marcogliese 2005 for reviews).

The anadromous allis shad *Alosa alosa* (Linnaeus, 1758) and twaite shad *Alosa fallax* (Lacépède, 1803) spend most of their life along the European Atlantic coast before returning in fresh waters to spawn generally in their natal rivers, with one spawning migration for *A. alosa* but several for *A. fallax* (for reviews: Baglinière and Elie 2000; Aprahamian et al. 2003a; Jolly et al. 2012). During the last decades, these closely related clupeids greatly declined in abundance throughout their geographic range, probably due to anthropogenic impact (e.g. overfishing, pollution, dam constructions, gravel extraction) (Baglinière and Elie 2000; Aprahamian et al. 2003a). Therefore, they are considered a vulnerable species (listed in Annex II of the EU Habitats Directive and Annex III of the Bern Convention) and are classified as of « least concern » by IUCN (Freyhof and Kottelat 2008ab). Up to now, respectively 23 (19 helminths and four crustaceans) and 20 (17 helminths and three crustaceans) taxa of metazoan parasites have been recorded in *A. alosa* and *A. fallax*, among them 11 shared by the two host species (Aprahamian 1985; Doherty and McCarthy 2002; Aprahamian et al. 2003a; Nunn et al. 2008; Bao et al. 2015ab). Numerous studies have been conducted in order to increase knowledge on shads for conservation purposes (e.g. Baglinière and Elie 2000; Aprahamian et al. 2003ab; Baglinière et al. 2003; Jolly et al. 2012; Acou et al. 2013; Martin et al. 2015; Mota et al. 2015), but the significance of parasites has rarely been considered and never included in management and conservation programs.

Parasite communities tend to be similar in hosts that are geographically, phylogenetically, ecologically and developmentally close from one another (Locke et al. 2013). *A. alosa* and *A. fallax* are closely related species between which extensive hybridization can occur despite assessment of independent lineages (Alexandrino et al.

2006) and some ecological and biological differences (Baglinière and Elie 2000; Taverny and Elie 2001ab; Aprahamian et al. 2003ab). Therefore, similarities and dissimilarities of the parasite communities are expected between *A. alosa* and *A. fallax*, depending on developmental stage, geographical site, and marine or freshwater phase. The analysis of metazoan parasite communities is thus undoubtedly an interesting and attractive way of getting knowledge on the spatial ecology of both shad species throughout their anadromous life-cycle as well as on their health status.

In this context, our objectives are:

(1) To describe and compare communities of metazoan parasites in *A. alosa* and *A. fallax* sampled in European Atlantic coastal-estuarine waters and rivers during their oceanic growth and anadromous breeding phases, and thus to determine how the parasitofauna of shads is influenced by environmental (salinity, site) and host physiological parameters (developmental stage, sex);

(2) to evaluate the impact of metazoan parasites on *A. alosa* and *A. fallax* using four condition indices (total weight, girth, fat content, and Fulton's K) as a proxy of fitness (Jakob et al. 1996);

(3) to determine if some parasite taxa could be used as biological tags to discriminate shad individuals (at a specific and/or population level) and/or to provide information on feeding habits and displacements.

Materials and Methods

Study-sites and fish samplings

The European "Natura 2000" networking program (Acou et al. 2013), which aims to increase knowledge on shad species for conversation purposes, provided a total of 96 *A. alosa* and 78 *A. fallax*. These were caught between May 2010 and March 2012 by professional fishermen in four French freshwater river systems (Vire, Vilaine, Loire, and Dordogne) and four estuarine and coastal waters from North-East Atlantic including Bay of Biscay and North Sea (Fig. 1, Table 1). Three sampling sites were found to harbor *A. alosa* and *A. fallax* in sympatric conditions (i.e. Loire, North Biscay Bay, and Adour).

Fish measurements

Total weight (TW, g), fork length (FL, mm) and girth (G, mm) of each fish were measured. Then, gonads were extracted and weighted (GW, g) in order to calculate the gonado-somatic ratio (= GW / TW) as a proxy of maturity stage and reproductive potential. The fat content in fish was proximately determined through the elemental bulk tissue carbon to nitrogen ratio (C/N) in muscles, calculated through stable isotope ratio

analyses by mass spectrometry. The use of this index relies on the assumption that an increase in tissue total lipid concentration correlated with increases in C/N ratios since lipid contains mainly carbon and few-to-no nitrogen (Barnes et al. 2007). The Fulton's K was used as a condition index for *A. alosa* and *A. fallax* because of their quasi-isometric length-weight growth (Bolger and Connolly 1989; Taverny and Elie 2001a; Correia et al. 2001) and was calculated as $K = 10^5 \ TW/FL^3$. Among the primary condition factors based on length-mass relationships, Fulton's K has previously been documented to be the best predictor of parasite load in *Lepomis macrochirus* Rafinesque, 1819 (Centrarchidae), significantly correlated with non-polar lipid density (Neff and Cargnelli 2004).

Whatever fish species, most individuals sampled were at an adult stage (84.4% for *A. alosa* and 68.0% for *A. fallax*) with a sex ratio in favor of females (58.0% and 79.3% respectively for *A. alosa* and *A. fallax*) (Table 2). Immature shads of both *Alosa* species were mostly captured in coastal waters during their growth phase (80.0% for *A. alosa* and 96.0% for *A. fallax*) (Table 2). Adults of *A. alosa* were mainly sampled in freshwaters (74.1%) whereas those of *A. fallax* were similarly sampled whatever the salinity (41.5% in freshwaters) (Table 2).

Parasitological research

All the 174 fish were frozen before the search for metazoan parasites as in previous studies (Gérard et al. 2013, 2015, 2016; Dessier et al. 2016). The following organs and tissues: skin, gills, muscles, heart, digestive tract, gonads, and body cavity were meticulously dissected under a binocular stereomicroscope. All the metazoan parasites found were numbered per organ and per fish, and morphologically identified to the species level excepted for nematodes. Morphological identification referred to Price (1961) for Monogenea, Bray and Gibson (1980) and Gibson and Bray (1986) for Digenea, Kuchta et al. (2005) for Cestoda, Golvan (1969) for Acanthocephala, Fagerholm (1991) and Anderson et al. (2009) for Nematoda, Kabata (1964) for Copepoda, Rushton-Mellor and Boxshall (1994) for Branchiura, Trilles (1975) for Isopoda, and Taverny and Elie (2010) for Petromyzontidae. Some nematode specimens (i.e. *Anisakis* Dujardin, 1845 and *Hysterothylacium* Ward and Magath, 1916) were preserved in 70% ethanol for molecular identification at the species level (see below).

The parasitological parameters used to describe the parasite community structure were: prevalence (P, number of hosts infected with a particular parasite species / number of hosts examined), taxa richness (number of parasite taxa infecting a host species), and abundance (number of individuals of a particular parasite species in/on a single host regardless of whether or not the host is infected) (Bush et al. 1997).

Molecular identification of nematodes via DNA sequencing

A total of 54 *Anisakis* and 249 *Hysterothylacium* were analyzed by molecular identification tools. DNA of each individual parasite was extracted using Wizard® Genomic DNA Purification Kit (Promega) following manufacturer's instructions after grinding of the parasite with a sterile piston pellet. DNA was kept at -20 °C until used. The mitochondrial cytochrome c oxidase subunit II (*cox2*) gene was amplified using the primers 211 F (5′-TTT TCT AGT TAT ATA GAT TGR TTY AT-3′) and 210 R (5′-CAC CAA CTC TTA AAA TTA TC-3′) as described in Nadler and Hudspeth (2000) and Valentini et al. (2006). Amplification of the nuclear rDNA region comprising ITS-1, 5.8S, and ITS-2 sequences was carried out with the primers NC5 (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) as described in Zhu et al. (1998). Automated DNA sequencing was performed by Genoscreen (Lille, France). Sequences were analyzed using BioEdit software to obtain consensus sequences from forward and reverse sequences. They were subsequently aligned with available *cox2* rDNA or ITS sequences for members of Anisakidae and Raphidascarididae by GenBank Blast software (Basic Local Alignment Search Tool) and Clustal X (Altschul et al. 1997).

Statistical analysis

All statistical analyses were performed using R software (R Core Team 2014) and packages 'vegan' (Oksanen et al. 2016), 'car' (Fox and Weisberg 2011), 'RVAideMemoire' (Hervé 2016) and 'lsmeans' (Lenth 2016).

Mean number of parasite taxa was compared between (i) all individuals, (ii) immatures and (iii) adults of the two host species using likelihood ratio tests applied on Generalized Linear Models (family: Poisson, link: log) (GLMs). The same procedure was used separately for each host species to test for an effect of developmental stage (immature vs adult), sex of adults (female vs male), salinity (river vs coastal/estuarine water) and site (with factor site nested into factor salinity).

In subsequent quantitative analyses, parasite taxa with low prevalence (< 6%) in both fish species [*Parahemiurus merus* (Linton, 1910), *Pomphorhynchus laevis* Müller, 1776, *Anilocra physodes* (Linnaeus, 1758), *Argulus foliaceus* (Linnaeus, 1758) and *Petromyzon marinus* Linnaeus, 1758] were not considered in analyses performed separately for each parasite species because of their rarity and probable limited impact on host populations.

Mean total abundance of parasites was compared between (i) all individuals, (ii) immatures and (iii) adults of the two host species using a Student *t*-test. Prevalence and abundance of each of the six common parasite taxa [*Mazocraes alosae* (Herman, 1782), *Hemiurus appendiculatus* (Rudolphi, 1802), *Hysterothylacium* sp., *Anisakis* spp., *Eubothrium fragile* (Rudolphi, 1802) and *Clavellisa emarginata* (Krøyer, 1837)] were compared in the same way (i.e. all individuals, immatures and adults) using likelihood ratio tests and ANOVAs, respectively.

Likelihood ratio tests were based on Generalized Linear Models (family: binomial, link: logit) (GLMs) whereas ANOVAs were based on Linear Models (LMs). In models focused on immatures and adults, the only explanatory variable was the host species. In models analyzing all individuals, explanatory variables considered were the host species, the sympatry (yes/no) and the interaction between these two factors. Chi-square tests and Student *t*-tests were performed to compare prevalence and abundance of the six common parasite taxa between hosts separately for each of the sympatric sites (Loire, Adour, and North Biscay Bay). A permutational F-test based on a Canonical Correspondence Analysis (CCA) was used to compare the distribution pattern of parasitofauna between host species, globally and in each of the three sympatric sites (Legendre and Legendre 2012).

Likelihood ratio tests based on GLMs (same family and link function as above), ANOVAs based on LMs, Student *t*-tests and permutational F-tests based on CCAs were also used to compare prevalence, abundance and distribution pattern of parasitofauna between (i) salinity and sites (with factor site nested into factor salinity), (ii) sexes (females vs males) and (iii) developmental stages (immatures vs adults), for the whole parasitofauna and for each of the major parasite taxa (i.e. prevalence > 10%), separately for each host species.

The relationship between host size and parasite prevalence and abundance was tested using Wald tests (based on GLMs for prevalence, LMs for abundance), at the scale of the whole parasitofauna and for each of the major parasite taxa, separately for each host species. For a better model fit, total parasite abundance was log-transformed for both host species, as well as the abundance of *M. alosae H. appendiculatus*, and *Hysterothylacium* sp. in *A. alosa* and *M. alosae*, *H, appendiculatus*, *Hysterothylacium* sp., *Anisakis* spp. and *Pronoprymna ventricosa* (Rudolphi, 1891) in *A. fallax*. The relationships between host condition parameters (total weight, girth, C/N ratio, and Fulton's K) and parasite abundance were tested using F-tests based on LMs (in which host size was included as a covariate to control for the bias induced by host age), at the scale of the whole parasitofauna and for each of the major parasite taxa, separately for each host species.

Differences are considered statistically significant at $P < 0.05$. Throughout the following sections, data are reported as means ± standard error (SE).

Results

1. Composition of metazoan parasite community in *A. alosa* and *A. fallax*

Each of the 96 *A. alosa* and 78 *A. fallax* dissected was infected (total prevalence of 100%) by one to six metazoan parasite taxa among the 12 identified in the whole sampling (Table 3). The mean number of parasite taxa per fish was not different between *A. alosa* (3.2 ± 0.1) and *A. fallax* (3.4 ± 0.2), but the mean total abundance of parasites per fish was significantly higher for *A. alosa*, i.e. 167.2 ± 9.6 (range: 5-534), than for *A. fallax*, i.e. 111.5 ± 11.1 (range: 4-435) ($P < 0.001$). Some organs (i.e. muscles, heart and gonads) were not found infected. Among the 12 parasite taxa recorded, helminths were the best represented (eight taxa with prevalence up to 99.0%), in contrast with crustaceans (three species) and Petromyzontidae (one species) that rarely occurred (prevalence < 6%) (Table 3).

In *A. alosa*, the parasite community was composed of nine taxa, among them four major taxa with prevalence > 57% [in decreasing order: the dominant species *M. alosae* on the gills, *Anisakis* spp. at third larval stage in the body cavity, *Hysterothylacium* sp. at larval (third and fourth) and adult stages and *H. appendiculatus* both taxa in the digestive tract], and five rare taxa with prevalence < 3% (Table 3).

In *A. fallax*, the parasite community also comprised nine taxa including three major taxa with relatively high prevalence > 58% [in decreasing order: the dominant species *H. appendiculatus* in the digestive tract, then *M. alosae* on the gills, and *Hysterothylacium* sp. at larval (third and fourth) and adult stages in the digestive tract], three taxa with intermediate prevalence between 28% and 42% (third-stage larval *Anisakis* spp. in the body cavity, then *P. ventricosa* and *E. fragile* both in intestine and pyloric caeca), and three minor taxa with prevalence < 6% (Table 3).

Based on molecular identification, three nematode species were unambiguously identified, i.e., *Anisakis simplex* sensu stricto (Rudolphi, 1809), *Anisakis pegreffii* Campana-Rouget and Biocca, 1955, and *Hysterothylacium aduncum* (Rudolphi, 1802) (Table 4). *A. simplex* s.s. and *H. aduncum* were recorded in both shad species and in most sampling sites, whereas *A. pegreffi* was only found in *A. fallax* from Loire and Pertuis Charentais (Table 4).

2. Is the metazoan parasite community influenced by host developmental stage (juvenile vs adult) and sex?

- Alosa alosa

Differences occurred depending on the developmental stage of *A. alosa* (Table 5). The mean number of parasite taxa per fish was not significantly different between immatures (2.5 ± 0.3) and adults (3.3 ± 0.1), but the mean total abundance of parasites per fish was almost thrice higher in adults (185.9 ± 9.7 belonging to six taxa) vs immatures (66.2 ± 15.5 belonging to seven taxa) ($P < 0.001$). The distribution patterns of the parasitofauna also differed between adults and immatures (CCA: pseudo-F = 3.425, df1 = 1, df2 = 94, $P = 0.021$) (Table 5). Four parasite taxa were common to immatures and adults, i.e. the four major taxa (Table 5). Both prevalences and abundances were higher in adults (vs immatures) for *H. aduncum* and *Anisakis* spp. ($P \leq 0.010$), but not different whatever the developmental stage for *M. alosae* and *H. appendiculatus* (Table 5). Uncommon parasite taxa were rare in immatures (*E. fragile*, *C. emarginata* and *A. physodes*) as well as in adults (*P. merus* and *P. marinus*) (Table 5).

No differences occurred between males and females whatever the parasite descriptor, i.e. mean number of parasite taxa per fish, mean total abundance of parasites per fish, distribution patterns of the parasitofauna, and prevalences and abundances of each of the four major parasite taxa (Table 5). Two rare parasite species found in females (i.e. *P. merus* and *P. marinus*) were not recorded in males (Table 5).

- Alosa fallax

The mean total abundance of parasites per fish was similar in immatures (121.6 ± 24.2) and adults (108.8 ± 11.9), but the mean number of parasite taxa per fish was lower in immatures (2.7 ± 0.2) than in adults (3.8 ± 0.2) ($P = 0.016$), and the distribution patterns of the parasitofauna differed depending on the developmental stage of *A. fallax* (CCA: pseudo-F = 11.662, df1 = 1, df2 = 75, $P < 0.001$) (Table 6). Two rare species found in adults (i.e. *P. laevis* and *A. foliaceus*) were not recorded in immatures (Table 6). All the seven taxa of metazoan parasites described in immatures were also present in adults (Table 6). Prevalences of *M. alosae*, *Anisakis* spp. and *P. ventricosa* were higher in adults (vs immatures) ($P \leq 0.034$) as well as *P. ventricosa* abundance ($P = 0.016$), whereas abundances of *M. alosae* and *Anisakis* spp. were similar in immatures and adults (Table 6). In contrast, prevalence and the abundance of *H. appendiculatus* were lower in adults than in immatures ($P \leq 0.010$) (Table 6). Among the 26 immatures, two were young-of-the-year (fork length of 85.1 and 89.6 mm), sampled in the North Sea and infected only by *H. appendiculatus* (with respectively 27 and 6 parasites). No differences were recorded whatever the developmental stage in prevalences and abundances of *H. aduncum*, *E. fragile* and *C. emarginata* (Table 6).

No differences occurred between males and females whatever the parasite descriptor, i.e. mean number of parasite taxa per fish, mean total abundance of parasites per fish, distribution patterns of the parasitofauna, and prevalences and abundances of each of the main parasite taxa except for *E. fragile* which was more prevalent in females than in males ($P = 0.049$) (Table 6). Three rare parasite species found in females (i.e. *P. laevis*, *C. emarginata* and *A. foliaceus*) were not recorded in males (Table 6).

3. Is the metazoan parasite community of *Alosa* sp. influenced by site and salinity?

- Alosa alosa

Whatever the salinity, the mean number of parasite taxa per fish was not different, but the mean total abundance of parasites per fish was higher in fresh waters, i.e. 187.9 ± 9.7 (belonging to six taxa), than in coastal and estuarine waters, i.e. 127.6 ± 19.2 (seven taxa) ($P = 0.002$), and the distribution patterns of the nine parasite taxa differed according to the salinity (CCA: pseudo-F = 2.971, df1 = 1, df2 = 91, $P = 0.032$) (Table 5). *P. merus* was only recorded in fresh waters whereas *E. fragile* and the crustacean *C. emarginata* and *A. physodes* were only found in salt waters; these four species being rare in *A. alosa* (total prevalence ≤ 2%). Among the four main parasite taxa infecting *A. alosa*, *Anisakis* spp. was more prevalent and abundant in fresh vs coastal/estuarine waters ($P < 0.001$) (Table 5). No differences according to the salinity were detected in prevalences and abundances of *M. alosae*, *H. aduncum* and *H. appendiculatus* (Table 5).

The mean number of parasite taxa per fish was not different among the sites as well as the mean total abundance of metazoan parasites per fish, but the distribution patterns of the nine parasite taxa differed between sites (CCA: pseudo-F = 10.196, df1 = 3, df2 = 91, $P < 0.001$) (Table 7). Between four and seven parasite taxa were recorded depending on the site with a mean value of 5.0 ± 0.6 (Table 7). The four main taxa (total prevalence > 57%) were always present whatever the site, but with inter-site differences in their abundances ($P < 0.001$) and in prevalences of *Anisakis* spp., *H. aduncum* and *H. appendiculatus* ($P \leq 0.008$); prevalence of *M. alosae* ranging from 95 to 100% was not significantly different between sites (Table 7).

- Alosa fallax

Whatever the salinity, the mean number of parasite taxa and the mean total abundance of parasites per fish were not different, but the distribution patterns of the nine parasite taxa differed according to the salinity (CCA: pseudo-F = 18.414, df1 = 1, df2 = 71, $P < 0.001$) (Table 6). *P. laevis* was only recorded in fresh waters whereas *C. emarginata* and *A. foliaceus* were only found in salt waters; these three species being rare in *A. fallax*

(total prevalence ≤ 5%) (Table 6). Among the six main parasite taxa (total prevalence ≥ 28%), *Anisakis* spp. and *P. ventricosa* were more prevalent in fresh vs coastal/estuarine waters ($P \leq 0.032$), and also more abundant in case of *P. ventricosa* ($P < 0.001$) but not in case of *Anisakis* spp. (Table 6). In contrast, prevalences and abundances of *H. aduncum* and *E. fragile* were higher in coastal/estuarine vs fresh waters ($P \leq 0.043$) (Table 6). No differences according to the salinity were detected in prevalences and abundances of *H. appendiculatus* and *M. alosae* (Table 6).

As for *A. alosa*, the mean number of parasite taxa and the mean total abundance of parasites per fish were not different among the sites, but the distribution patterns of the nine parasite taxa differed between sites (CCA: pseudo-F = 6.074, df1 = 4, df2 = 71, $P < 0.001$) (Table 8). Between six and seven parasite taxa were recorded depending on the site with a mean value of 6.6 ± 0.2 (Table 8). The six more prevalent taxa of *A. fallax* (total prevalence > 28%) were always present whatever the site, except *H. aduncum* not recorded in the Dordogne river. Abundance varied between sites for *M. alosae*, *H. appendiculatus*, *Anisakis* spp. and *P. ventricosa* ($P \leq 0.039$), not for *H. aduncum* and *E. fragile*, whereas prevalence varied between sites for *M. alosae*, *Anisakis* spp., *P. ventricosa*, *H. aduncum* and *E. fragile* ($P \leq 0.034$), not for *H. appendiculatus* (Table 8).

4. Are metazoan parasite communities different between host species in the whole sampling, in sympatric conditions and depending on developmental stage?

Six parasite taxa (five helminth and one crustacean taxa) were common to *A. alosa* and *A. fallax* (Table 3), but with significant differences in their whole distribution patterns including in sympatric conditions (CCA: pseudo-F = 25.990, df1 = 1, df2 = 170, $P < 0.001$). Prevalences and abundances of shared parasite taxa were also significantly different between shad species ($P < 0.001$, Table 9), except for those of *H. aduncum* and the rare copepod *C. emarginata*, and also abundance of *H. appendiculatus* (Table 9). These differences and similarities were not influenced by sympatric conditions except for prevalence and abundance of *Anisakis* spp. ($P \leq 0.005$) and, in a lesser extent, abundance of *E. fragile*, a rare species in *A. alosa* ($P = 0.050$) (Table 9).

When considering each of the three sympatric sites (Tables 7, 8), differences between *A. alosa* and *A. fallax* were also observed in the distribution patterns of the six common parasite taxa in Loire and Adour (CCA: pseudo-F = 10.075 and 19.649 respectively, df1 = 1, df2 = 28 and 27, $P < 0.001$) where most shads were adult (98.3%) (Table 2), but not in the North Bay of Biscay where 60.0% of *A. alosa* and 43.8% of *A. fallax* were immature (Table 2). In this marine sympatric site, prevalences and abundances of the six shared parasite taxa

were not different in the two fish species, except prevalence of *H. aduncum* twice lower in *A. alosa* (40.0 ± 11.0%) vs *A. fallax* (81.3 ± 9.8%) ($P = 0.031$) (Tables 7, 8).

When considering the developmental stage, the mean number of parasite taxa was similar between immatures of *A. alosa* (2.5 ± 0.3) and *A. fallax* (2.7 ± 0.2), and between adults of the two shad species (respectively 3.3 ± 0.1 and 3.8 ± 0.2). The mean total abundance of parasites was also not significantly different between immatures of *A. alosa* (66.2 ± 15.5) and *A. fallax* (121.6 ± 24.2), whereas it was higher for adults of *A. alosa* (185.9 ± 9.7) than for adults of *A. fallax* (108.8 ± 11.9) ($P < 0.001$). Differences and similarities in prevalences and abundances of the six common parasite taxa between *A. alosa* and *A. fallax* were also affected by the developmental stage (Tables 5, 6 and 10). For immature shads, prevalences of *M. alosae*, *H. appendiculatus* and *H. aduncum* were different between *A. alosa* and *A. fallax* ($P \leq 0.040$), but not different in case of *Anisakis* spp., *E. fragile* and *C. emarginata* (Tables 5, 6 and 10). No differences occurred in the abundances of each of the six common parasite taxa between immatures of *A. alosa* and *A. fallax* (Tables 5, 6 and 10). When considering adult shads, prevalences and abundances of most shared parasite taxa were significantly different between the two fish species ($P \leq 0.009$), except for those of *H. aduncum* and *C. emarginata*, and also abundance of *H. appendiculatus* (Tables 5, 6 and 10).

5. Relationship between parasite abundance and fish size and impact of parasitism on host body condition

- Alosa alosa

A positive relationship was demonstrated between the total number of parasites and the fork length of *A. alosa* (Wald test: $t = 8.568$, df = 94, $P < 0.001$). Among the four major parasite taxa of *A. alosa*, prevalences and abundances of both *H. aduncum* and *Anisakis* spp. and abundance of *M. alosae* were positively correlated to the fork length of *A. alosa* ($P \leq 0.021$); no relationship was detected for prevalence of *M. alosae* and both prevalence and abundance of *H. appendiculatus*.

Independently of fish size, the whole parasite abundance was positively related to the following body condition indices: girth, C/N ratio, and Fulton's K ($P \leq 0.046$); the positive relationship was not significant with the total weight of shads.

When considering each of the main parasite taxa, a positive relationship was demonstrated between *H. aduncum* abundance and each body condition index (total weight, girth, C/N ratio, and Fulton's K) ($P \leq 0.026$), whereas a negative relationship occurred between *H. appendiculatus* abundance and both total weight and Fulton's K ($P \leq$

0.007), not with girth and C/N ratio. No significant relationship was evident for *M. alosae* and *Anisakis* spp. whatever the body condition index considered.

- Alosa fallax

The positive relationship between the total number of parasites and the fork length of *A. fallax* was not significant. Among the six main parasite taxa, prevalences and abundances of *M. alosae*, *Anisakis* spp. and *P. ventricosa* were positively related to the fork length of *A. fallax* ($P \leq 0.007$), whereas prevalence and abundance of *H. appendiculatus* were negatively related to the fork length ($P = 0.027$ and < 0.001 respectively); no significant relationship with the fish length was demonstrated for prevalences and abundances of *H. aduncum* and *E. fragile*.

Independently of fish size, the whole parasite abundance was negatively related to the girth ($P = 0.034$); no significant relationship was detected with the other body condition indices (total weight, C/N ratio, and Fulton's K).

When considering each of the six main parasite taxa, some of them (*H. aduncum*, *P. ventricosa*, and *E. fragile*) were correlated to the body condition of *A. fallax*. Indeed, a positive relationship was demonstrated between *H. aduncum* and both total weight and Fulton's K ($P \leq 0.013$), as well as between *E. fragile* and both girth and Fulton's K ($P \leq 0.007$). In contrast, the relationship was negative between *P. ventricosa* and fish total weight, girth and Fulton's K ($P \leq 0.010$). No relationships were found with *H. appendiculat*us, *M. alosae*, and *Anisakis* spp. whatever the body condition index considered.

Discussion

1) High significance of metazoan parasites in *A. alosa* and *A. fallax* and comparison with previous parasitological studies

Our study highlights the importance of metazoan parasites in *A. alosa* and *A. fallax* in terms of their total prevalence of 100% and their high mean abundance (respectively 167 ± 10 and 112 ± 11 parasites per fish) and diversity (nine parasite taxa per host species). Among the 12 parasite taxa registered, four were new records, all at an adult stage and with low prevalences (< 3%), corresponding to i) three opportunistic hematophagous ectoparasites: the crustaceans *A. foliaceus* and *A. physodes*, and the anadromous jawless vertebrate *P. marinus* (Pasternak et al. 2000; Taverny and Elie 2010; Smit et al. 2014), and ii) the generalist trophically-transmitted digenean *P. merus* reported in various families of pelagic teleosts (Gibson and Bray 1986).

Taxa different from helminths were rare in shads except the copepod *C. emarginata*, a gill parasite specific to the genus *Alosa* (Kabata 1964). We only recorded *C. emarginata* in coastal/estuarine sites, and mainly on immatures (prevalence of 7 ± 6% and 12 ± 7% respectively for *A. alosa* and *A. fallax* vs 0% and 2 ± 2% for adults). Our results are in line with those of other studies (Tables 11, 12), suggesting that *C. emarginata* is a marine ectoparasite able to tolerate fresh waters, with *A. fallax* as preferred host compared to *A. alosa*.

Both shad species were highly parasitized by helminths with six and seven taxa respectively in *A. alosa* and *A. fallax* among them five in common. The most prevalent taxa were the monogenean *M. alosae* (dominant species for *A. alosa*), the digeneans *H. appendiculatus* (dominant species for *A. fallax*) and *P. ventricosa* (only recorded in *A. fallax*), the cestode *E. fragile* (rare in *A. alosa*), and the nematodes *Anisakis* spp. (i.e. *A. simplex* s.s., *A. pegreffii*) and *H. aduncum*. The shads were mainly used as definitive hosts by helminths (except *Anisakis* using shads as paratenic hosts) and infected following ingestion of parasitized preys (except *M. alosae* actively infecting host gills) (Table 3).

Previous parasitological data on *A. alosa* and *A. fallax* are rare and tend to be contrasted depending on developmental stage, partly due to the anadromous life cycle inducing changes in diet and habitat use (for reviews: Baglinière and Elie 2000; Aprahamian et al. 2003a), and more generally, due to increasing probability of meeting parasites over time/with age (Dogiel et al. 1958).

Only one metazoan parasite taxon (i.e. the gill monogenean *Gyrodactylus* von Nordmann, 1832 commonly infecting freshwater fish) has previously been reported in larvae of *A. fallax*, and four taxa [i.e. *Gyrodactylus* and three trophically-transmitted helminths: the acanthocephalan *P. laevis* and larval nematodes *Spinitectus* Fourment, 1883 and Proleptinae (Schulz, 1927)] in 0+ immatures from the English Wye river and Towy estuary (Nunn et al. 2008). In our study, the youngest shads were two 0+ immatures of *A. fallax* from the North Sea, only parasitized by *H. apppendiculatus* (recorded in 100% of immatures and 83% of adults), typically found in the stomach of marine teleosts preying planktonic copepods and chaetognaths (Chiriac and Udrescu 1973; Gibson and Bray 1986). According to Aprahamian (1985), shad infection probably occurs in marine and estuarine waters during the growth phase.

In our study, each immature of *A. fallax* harbored between one and five metazoan parasite taxa among seven taxa, but none of them described by Nunn et al. (2008). Nevertheless, *P. laevis*, a freshwater parasite acquired by various teleosts that preyed amphipods (Kennedy 2006), is sometimes recorded in adults of *A. fallax* (Table 12). The absence of overlap between parasite communities of immature shads may be due to i) age (larvae, 0+ and older immatures) resulting in potential differences in habitat and food use (Baglinière and Elie 2000), ii) inter-

site prey availability inducing differences in the euryphagous and opportunistic diet of immatures (Nunn et al. 2008; Baglinière and Elie 2000), iii) absence of host species needed to complete the heteroxenous life cycle of most helminths, and/or iv) potential mortality of infected immatures as shown for *Gyrodactylus* (Grano-Maldonado et al. 2011).

Unlike immatures shads, the parasitic diversity in adults from our study was on the same order than in other parasitological investigations, i.e. between six and nine taxa, with a general overlap of the parasitofauna (Tables 11, 12; Aprahamian 1985; Doherty and McCarthy 2002; Bao et al. 2015ab). In *A. alosa*, our main parasite taxa (i.e. *M. alosae*, *H. appendiculatus*, *Anisakis* spp. and *H. aduncum*) were also recorded in Western Iberian rivers with almost similar prevalences and abundances on the whole, whereas *Anisakis* spp. was not recorded in Irish Barrow river and Waterford estuary despite comparable prevalences and abundances for the three other taxa (Table 11). In *A. fallax*, parasite communities were more contrasted than in *A. alosa* depending on investigation (Table 12). All the seven taxa in English Severn river and estuary, also recorded in Irish waters, occurred in our study, but with some differences as for *P. ventricosa*, extremely abundant, and *Anisakis* spp., totally absent, in English and Irish waters (Table 12). In Iberian rivers, *A. fallax* was differently parasitized compared to other studies and was never infected by *M. alosae*, *E. fragile* and *P. ventricosa* (Table 12).
These results (Tables 11, 12) confirm that *A. alosa* is the preferred host of *M. alosae* compared to *A. fallax* in North-East Atlantic and connected rivers (Gérard et al. 2016), the life cycle of the monogenean being closely synchronized with those of its host (Bychowsky 1957). Data also suggest a decreasing occurrence from North to South of *E. fragile* and *P. ventricosa*, gut parasites preferentially infecting *A. fallax* (vs *A. alosa*). This latitudinal gradient may be related to the availability of intermediate hosts preyed by the shads, i.e. marine planktonic copepods for *E. fragile* (Kennedy 1981) whereas amphipods for *P. ventricosa* (Bray and Gibson 1980). Moreover, the absence of *Anisakis* spp. in English and Irish waters is questioning since *A. simplex* s.s. is widespread between 35°N and Arctic Polar Circle whereas the upper limit of *A. pegreffii* is the Iberian coast of North-East Atlantic (Mattiucci and Nascetti 2006). Our record of *A. pegreffii* in *A. fallax* between 45°N and 47°N (Loire, Pertuis Charentais) however suggests that its upper geographical limit might be higher than Iberian waters. Further studies are needed to explore *A. pegreffii* distribution in North-East Atlantic and to understand why no *Anisakis* spp. was found in shads from English and Irish waters.

2) Low variability of metazoan parasite communities according to physiological characteristics (developmental stage, sex) and environmental conditions (salinity, geographical area)

The presence or absence of parasites within host populations is the result of a complex of abiotic and biotic factors , therefore a great variability of parasitofauna is usually observed from one host population to the next for most fish species (Anderson and Sukhdeo 2010; Campbell et al. 2007; Kennedy 2009; Kleinertz et al. 2012; Gérard et al. 2013). Surprisingly, metazoan parasite communities of *A. alosa* and *A. fallax* remained stable in their composition whatever host physiological characteristics and environmental conditions. In particular, parasitofauna was highly similar whatever the sex despite absence in males of some rare species recorded in females. When considering the other intrinsic (host developmental stage) and extrinsic (sample site, salinity) factors, our results emphasized only limited quantitative differences in prevalences, abundances and distribution patterns of parasites.

• Influence of host developmental stage and salinity on parasite communities

For *A. alosa*, the four major parasite taxa were always found whatever developmental stage and the richness of parasite taxa was unchanged between immatures and adults. Adults harbored a thrice higher abundance of parasites compared to immatures, partly explained by a higher carrying capacity of adults (1.6 times greater body size compared to immatures), but also indicating parasite accumulation which is a common time-driven process (Dogiel et al. 1958). This parasite accumulation with size/age mainly corresponded to *Anisakis* spp. and *H. aduncum* much more prevalent and abundant in adults than in immatures, whereas the distribution patterns of *M. alosae* and *H. appendiculatus* were similar whatever developmental stage. The increased parasitism with host size/age underlined that adults of *A. alosa*, although more heavily infected by *Anisakis* spp. and *H. aduncum* than immatures, suffered no acute pathogenicity as shown by the absence of effect of these nematodes on *A. alosa* body condition.

The occurrence of shared trophically-transmitted *H. appendiculatus*, *Anisakis* spp. and *H. aduncum* demonstrated that both immatures and adults preyed similar parasitized invertebrates (mainly crustaceans and chaetognaths) and small fish (Table 3). Moreover, the increasing prevalence and abundance of *Anisakis* spp. and *H. aduncum* with *A. alosa* size/age suggested an increasing consumption of preys infected by these nematodes from immature to adult stage. Parasite species unshared by both developmental stages were rarely recorded in *A. alosa* (prevalence $\leq$ 3%), i.e. ectoparasites with active transmission (*C. emarginata*, *A. physodes*, and *P. marinus*), and *E. fragile* and *P. merus*, trophically-transmitted via copepods and chaetognaths as for most shared helminths (Table 3).

As most immatures of *A. alosa* were sampled in coastal/estuarine waters whereas in rivers for most adults due to anadromous semelparous life history, similarities/differences between immatures and adults tend to reflect those depending on salinity. Indeed, whatever salinity and developmental stage, the mean number of parasite taxa, and prevalences and abundances of *M. alosae* and *H. appendiculatus* were unchanged; in rivers as in adults, the mean total abundance of parasites was higher as well as *Anisakis* spp. prevalences and abundances. The single difference implied *H. aduncum* for which prevalence and abundance were unchanged whatever salinity (whereas higher for adults vs immatures). Moreover, all the four major helminth taxa infecting *A. alosa* appear euryhaline because of their high prevalences in marine (57-100%) and fresh (59-98%) waters.

For *A. fallax*, all the seven parasite taxa infecting immatures mostly captured in coastal/estuarine waters also occurred in adults, suggesting high similarity in parasitofauna composition whatever developmental stage, despite some differences in their distribution patterns. Only two freshwater species, *P. laevis* and *A. foliaceus*, rare in adults (prevalence < 2%), were not recorded in immatures. The mean number of parasite taxa was higher in adults than in immatures, suggesting an increasing probability to meet more diverse parasites over time. Surprisingly, in contrast to *A. alosa*, despite the 1.4 times greater body size of *A. fallax* adults (vs immatures) and the general time-dependent parasite accumulation (Dogiel et al. 1958), the mean total abundance of parasites was similar whatever developmental stage, and the positive relationship between parasite abundance and host size was not significant. These contrasting results can be explained when considering each parasite taxa of our study. Indeed, the abundance of *M. alosae*, *Anisakis* spp. and *P. ventricosa* in *A. fallax* was positively related to host size whereas negatively for *H. appendiculatus* (not demonstrated for *A. alosa*), thus indicating differences in host-parasite interactions depending on parasite taxa and host species. Nevertheless, the similar parasite abundance in immatures and adults of *A. fallax* potentially suggests i) the death of the most heavily infected adults, ii) the existence of regulation processes limiting parasite number to reach an equilibrium between host health preservation and spatial and energy needs of parasites, and/or iii) the death of stenohaline parasites in iteroparous spawning adults due to repeated migrations (up to seven) between marine and fresh waters (Aprahamian et al. 2003a for review).

The occurrence of five trophically-transmitted helminths shared by *A. fallax* immatures and adults implied resemblances in their diet (as also found for *A. alosa*). Indeed, similar prevalences and abundances of *H. aduncum* and *E. fragile* whatever developmental stage revealed similar consumption of infected preys, i.e. various crustaceans, chaetognaths and small fish (Table 3). However, differences in prevalences and abundances of *H. appendiculatus*, *Anisakis* spp., and *P. ventricosa* suggest that compared to *A. fallax* adults, immatures

preyed a greater proportion of planktonic crustaceans and chaetognaths infected by *H. appendiculatus* and/or *Anisakis* spp., but a smaller proportion of amphipods infected by *P. ventricosa* (Table 3). As for *A. alosa*, further thorough investigations would be necessary to know exactly what preyed host species are implied in the contamination of *A. fallax* immatures and adults.

In contrast to *A. alosa*, differences/similarities depending on developmental stage of *A. fallax* did not necessary reflect those depending on salinity because of repeated spawning migrations between rivers and marine waters (Aprahamian et al. 2003a). The mean total abundance of parasites was similar whatever salinity and developmental stage, whereas the mean number of parasite taxa was similar whatever salinity but different between immatures and adults. Among the seven parasite taxa shared by both developmental stages, only *C. emarginata* was not recorded in rivers, confirming its marine preference evoked previously. The six other taxa (helminths with prevalence > 28%) occurred whatever salinity, thus were probably euryhaline (as suggested for major parasite taxa of *A. alosa*) or at least able to tolerate some salinity fluctuations due to repeated spawning migrations. It was probably the case of *M. alosae* and *H. appendiculatus* similarly abundant and highly prevalent (≥ 80%) both in coastal/estuarine and fresh waters (whereas differently depending on developmental stage). Despite their probable tolerance to salinity fluctuations, the other major helminths infecting *A. fallax* had different distribution patterns according to salinity. *H. aduncum* and *E. fragile* were more prevalent and abundant in marine waters (no differences according to developmental stage) where crustaceans, chaetognaths, and fish infected by these parasites (Table 3) were probably more available, and thus, preyed during repeated marine stays of *A. fallax*. In contrast, *P. ventricosa* was more prevalent and abundant in rivers (as well as in adults) than in marine waters, revealing an increased ingestion of infected amphipods (Table 3) in fresh waters, and also feeding of adults during migration to spawning grounds. Surprisingly, despite marine preference (Mattiucci and Nascetti 2006, 2008), *Anisakis* spp. was similarly abundant whatever salinity, but more prevalent in fresh waters (as in adults). Such difference was also observed for *A. alosa* for which *Anisakis* spp. was not only more prevalent, but also more abundant in fresh (vs marine) waters. These results reveal the complexity of all interacting abiotic and biotic factors including availability of food resources, feeding habits and habitat use of the shads, and subsequent parasitic contamination depending on freshwater or marine phase. It also suggests the possibility of being infected by *Anisakis* spp. in rivers, and not only in marine waters with potential epidemiological consequences.

• Influence of sample site on parasite communities

Whatever shad species, both mean number of parasite taxa and mean total abundance of parasites per fish were comparable between sampling sites. The unchanged mean abundance of parasites per fish whatever site suggests the existence of density-dependent regulation constraining establishment of the parasite infracommunity in its host (and thus exploitation of host resources by parasites) to a threshold in such a way that the carrying capacity of infected host individual is not exceeded by parasites (equilibrium) (Combes 1995; Kennedy 2009; Hechinger 2013 for reviews). Moreover, the main parasite taxa infecting *A. alosa* and *A. fallax* (prevalence > 28%) were always present whatever site (except *H. aduncum* not recorded in *A. fallax* from the Dordogne river), with some differences in prevalences and abundances. It suggests stable, predictable, and structured parasite component communities in both shad species as shown for some percids (Carney and Dick 1999; Nelson and Dick 2002; Zelmer 2014), rather than non-equilibrial, stochastic assemblages as described for helminth communities of most freshwater fish (Kennedy 2009 for review).

3) High similarity in metazoan parasite communities of *A. alosa* and *A. fallax*

Metazoan parasite communities of the closely related *A. alosa* and *A. fallax* were highly similar in their composition and shared six taxa corresponding to five helminths and the copepod *C. emarginata*. The mean number of parasite taxa harbored per fish was not different between host species, but the total abundance of parasites per fish was 1.5 times higher in *A. alosa* than in *A. fallax*, potentially resulting from a size effect that enable larger fish to acquire more parasites than smaller counterparts (Zelmer 2014).

The distribution patterns of some parasite taxa shared by *A. alosa* and *A. fallax* could be different between host species, including in sympatric conditions. However, the four most prevalent parasites (≥ 42%) among the nine taxa recorded in each host species were always *M. alosae*, *H. appendiculatus, Anisakis* spp. and *H. aduncum*, reflecting ecological and physiological resemblances between *A. alosa* and *A. fallax*.

First, the high occurrence of *M. alosae* in semelparous *A. alosa* (99%, preferred host) and iteroparous *A. fallax* (82%) revealed similarities in their reproductive behavior with some overlap of their spawning grounds and season. Indeed, egg deposition of *M. alosae* on host gills coincided precisely with the pre-spawning period of shads (Bychowsky 1957).

Second, similarities in *A. alosa* and *A. fallax* feeding ecology occurred since three of the four most prevalent shared taxa were trophically-transmitted. Both shad species are considered euryphagous and opportunists at all life stages, using a broad range of trophic resources in upstream and estuarine/marine environments, fish being more consumed by *A. fallax* than *A. alosa* (e.g. Assis et al. 1992; Baglinière and Elie 2000; Taverny and Elie

2001b; Correia et al. 2001; Aprahamian et al. 2003a; Maitland et al. 2005; Nunn et al. 2008; Ceyhan et al. 2012; Mota and Antunes 2012; Skóra et al. 2012; Nachón et al. 2013). Therefore, along their life, shads are susceptible to ingest a myriad of preys including invertebrates and fish infected by *H. appendiculatus, Anisakis* spp. and *H. aduncum* (Table 3).

When considering developmental stage, similarities in the parasitofauna were greater between immatures than between adults of the two shad species. Indeed, for immatures, abundances of each of the six shared parasite taxa and prevalences of *Anisakis* spp., *E. fragile* and *C. emarginata* were similar whatever shad species, whereas for adults, prevalences and abundances of most shared parasite taxa were different between *A. alosa* and *A. fallax*. Moreover, in the single marine sympatric site of North Biscay Bay where most sampled shads were immature, no differences were detected between shad species whatever parasite descriptor, except *H. aduncum* prevalence twice higher in *A. fallax* compared to *A. alosa*. This difference may be due to a preferential consumption by *A. fallax* of anchovies, whereas accidental for *A. alosa*, in Biscay Bay (Taverny and Elie 2001b) where 90-100% of anchovies were found infected by third-larval *H. aduncum* (Dessier et al. 2016). The parasitism of *P. ventricosa* and *E. fragile*, preferentially infecting *A. fallax*, but absent or rare in *A. alosa* at an adult stage (Tables 11, 12), also suggested that amphipods and copepods, respective intermediate hosts of these helminths (Table 3), were more frequently preyed by *A. fallax* than by *A. alosa*. Further studies are needed to explore if some of these trophically-transmitted helminths could be used as biological tags to discriminate shad species.

Thus, despite overall parasite similarity, our data underline increasing differences with the age between shad species, in particular in their diet, mainly occurring after sexual maturity in relation with semelparous vs iteroparous reproductive strategy (Baglinière and Elie, 2000; Aprahamian et al. 2003a for reviews), but also probably due to a variety of other abiotic and biotic factors.

Similarity in metazoan parasite communities between host species is not only resulting from similarities in reproductive and/or feeding ecology, despite ecological convergence of host species being a key factor in determining the extent to which they share parasites (Poulin 2010). For freshwater fish, the strongest predictors of parasite community similarity are the genetic distance between host species and their patterns of habitat use, while diet, trophic level, size and spatial proximity of hosts showed relatively little association with parasite community similarity (Locke et al. 2013). High similarity was thus shown for parasite communities of sister species, Eurasian *Perca fluviatilis* Linnaeus, 1758 and North American *Perca flavescens* (Mitchill, 1814), despite their geographical isolation for millions of years (Carney and Dick 1999). When considering marine fish, helminth parasites are often generalists, lacking host specificity for intermediate and definitive hosts, and many

possess long-lived larvae residing in intermediate and paratenic hosts (Marcogliese 2002 for review). These properties are believed i) to be adaptations to long food chains and low densities of organisms distributed over broad spatial scales characterizing open marine systems, and ii) to lead to homogenization of parasite communities among fish species (Marcogliese 2002).

Concerning the closely related anadromous *A. alosa* and *A. fallax*, both their ecological convergence and phylogenetic history (Baglinière and Elie 2000; Aprahamian et al. 2003a; Jolly et al. 2012; Alexandrino et al. 2006; Faria et al. 2012) seem responsible for the overall high similarity of metazoan parasite communities.

4) Fitness loss induced by metazoan parasites in *A. alosa* and *A. fallax*

Parasites with complex life cycles reliant on predation-mediated transmission (as most helminths recorded in *Alosa* spp.) are generally associated with higher mortality risk than those exploiting other transmission strategies (Robar et al. 2010). Therefore, parasitism is considered an additional stress that may regulate host populations (e.g. Esch et al. 1997).

Surprisingly, most parasites we recorded in *Alosa* spp. seemed to have no lethal or sublethal effects on their host. Only *H. appendiculatus* and *P. ventricosa* were found to decrease the body condition of respectively *A. alosa* and *A. fallax*, revealing a host fitness loss induced by parasitism.

H. appendiculatus was highly prevalent in *A. fallax* (89%) and *A. alosa* (57%), occurring whatever site and developmental stage. As the mean abundance of *H. appendiculatus* was similar in both shad species, we expected a similar effect of this digenean on its host. Surprisingly, contrarily to *A. alosa* for which *H. appendiculatus* induced a decrease of fish weight and Fulton's K, no negative impact was demonstrated on the condition of *A. fallax*. *H. appendiculatus* being the dominant parasite species of *A. fallax*, we suppose that *A. fallax* is the preferred host species, having a probably long co-evolved and well co-adapted association with *H. appendiculatus*. Because greater pathogenicity is often observed for recent host-parasite associations (e.g. Kennedy 1994; Kania et al. 2010), the association between *H. appendiculatus* and *A. alosa* could be more recent, thus inducing a significant host fitness loss.

In case of *P. ventricosa* recorded in *A. fallax* (39%, 28 parasites per fish) but not in *A. alosa*, a negative impact was demonstrated on host total weight, girth and Fulton's K. This result suggests that *P. ventricosa* could represent a serious threaten for host shad populations, in particular for those with both high prevalences and abundances as observed in the Dordogne river (80%, 90 parasites per fish) or in English and Irish waters (Table 12).

Whatever host-parasite combination, parasites and their host compete for resources in such way that host survival and fecundity could be affected, even if no pathology is obvious and even if this effect may be drowned in the background noise of all other factors that affect these life-traits (Combes 1995; Thomas et al. 2007; Robar et al. 2010; McElroy and De Buron 2014 for reviews). As stated by Poulin (1998), a diversity of strategies for host exploitation has flourished among parasite taxa, such that parasites cause anything from undetectable to drastic changes in host fitness. For instance, the defence strategy of tolerance enables to limit health effects of parasites without preventing infection or controlling parasite replication (Råberg 2014). Otherwise, equilibrium between parasite virulence and host defence would not be expected to be disrupted until parasite prevalence reaches a threshold value, specific to each host population and each host-parasite association (Bouchut et al. 2008).

Thus, even if increased vulnerability and decreased fitness of infected shads are sometimes not obvious, it is crucial to better understand the highly complex host-parasite interactions and take into account the potential pathogen effects of parasites in shad conservation programs.

Conclusions

Our study demonstrates that during their oceanic growth and anadromous breeding phases in European Atlantic coastal-estuarine waters and rivers, *A. alosa* and *A. fallax* harbor stable metazoan parasite communities. All shads are parasitized whatever environmental and physiological conditions, and mostly by euryhaline and generalist trophically-transmitted helminths. Thus, shads contribute to parasite contamination of fresh and marine waters as shown in American rivers where *Anisakis* spp. is transmitted to marine but also freshwater mammals via infected shads (Shields et al. 2002).

As expected, metazoan parasite communities of *A. alosa* and *A. fallax* are highly similar, reflecting ecological convergence and phylogenetic history, but also suggest increasing dissimilarity with the age mainly related to differences in diet and reproductive strategy.

Information provided by trophically-transmitted helminths demonstrates the consumption of various invertebrates and small fish by *A. alosa* and *A. fallax*, both in fresh and marine waters and at immature and adult stages, and confirms the generalist and opportunist diet of both shad species demonstrated via trophic studies (see references cited above).

Although host fitness loss induced by parasites was not obvious except for two digenean species, metazoan parasites may negatively impact the condition of *A. alosa* and *A. fallax*, thus increasing their vulnerability at a

time when they greatly declined in abundance throughout their geographic range (Baglinière and Elie 2000; Aprahamian et al. 2003a). Because of their omnipresence and ecological significance, there is a dire need to integrate parasitic helminths in further multidisciplinary investigations to get knowledge on *A. alosa* and *A. fallax* and to develop efficient management and conservation programs.

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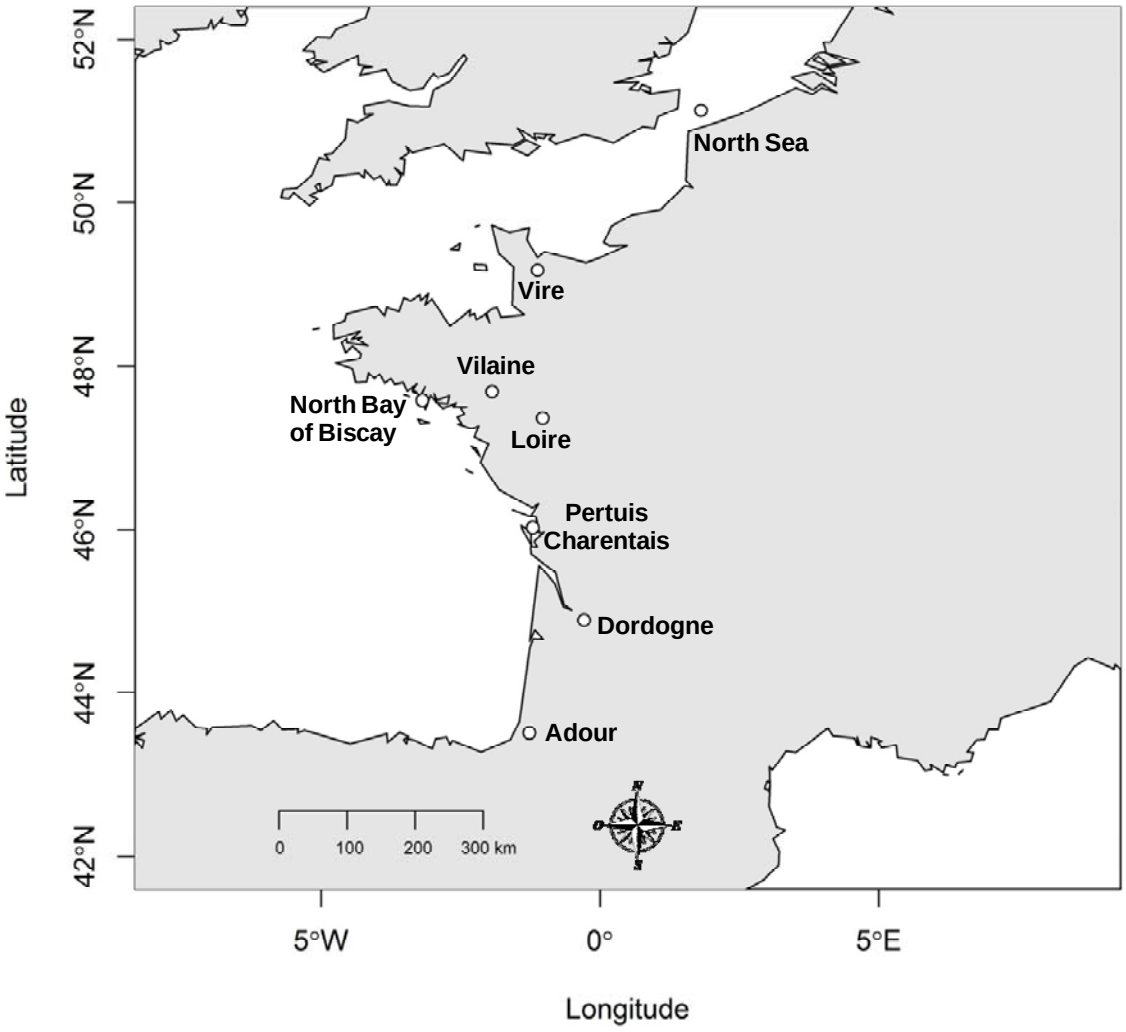

Fig 1 Geographical position of the sampling sites of *Alosa alosa* and *Alosa fallax* in four French rivers (Vire, Vilaine, Loire, and Dordogne) and four coastal-estuarine waters in North-East Atlantic (North Sea, North Bay of Biscay, Pertuis Charentais, and Adour). The map was generated using R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) with the marmap package (Pante and Simon-Bouhet 2013)

Table 1. Sampling rivers and coastal/estuarine waters of *Alosa alosa* (96) and *Alosa fallax* (78) in a latitudinal order (from North to South) with the ranges of salinity (g/l) and temperature (°C), and the dates of fish capture.

	Salinity	Temperature	Sampling dates
Rivers :			
Vire	0	10.0-20.0	18 May, 2 Jun
Vilaine	0	3.0-10.0	27 May, 1 Jul
Loire	1.0-6.0	12.0-19.0	1 Apr, 1-12 May, 7 Nov
Dordogne	0.1-0.3	13.0-21.0	1 Apr, 25 Apr, 1 May
Coast/estuaries :			
North Sea	34.6–34.8	6.3–10.9	15-24 Jan, 2-9 Feb
North Biscay Bay	30.1–35.6	5.9–21.8	13-27 Jan, 2 Feb, 1-16 Mar, 7 Apr, 2 Jun, 1-26 Aug, 1 Oct, 7 Nov, 16-20 Dec
Pertuis Charentais	31.7–34.8	7.5–19.7	1 Mar, 20 Aug
Adour	20.0-33.0	13.0-17.0	25-28 Apr, 1 May

Table 2. Number and mean fork length (FL ± SE, mm) of *Alosa alosa* and *Alosa fallax* (immatures, males, females) sampled in the 8 study-sites of North-East Atlantic coastal/estuarine waters and connected rivers.

	Alosa alosa					*Alosa fallax*				
Sites	Immatures	Males	Females	Total	FL ± SE	Immatures	Males	Females	Total	FL ± SE
Vire		11	10	21	484.3 ± 6.3					
Vilaine	3	5	12	20	489.5 ± 7.1					
Loire		4	18	22	493.2 ± 4.6	1		7	8	432.8 ± 15.9
Dordogne							2	13	15	388.8 ± 12.3
Rivers	3	20	40	63	489.1 ± 3.5	1	2	20	23	404.1 ± 10.5
North Sea						9	1	1	11	236.6 ± 27.0
North Biscay Bay	12	7	1	20	337.6 ± 19.1	7	6	3	16	389.6 ± 6.5
Pertuis Charentais						8	2	2	12	300.6 ± 19.5
Adour		7	6	13	471.8 ± 12.0			16	16	427.4 ± 4.2
Coast/estuaries	12	14	7	33	390.5 ± 17.0	24	9	22	55	350.6 ± 12.1
Total	15	34	47	96	455.2 ± 7.9	25	11	42	78	366.4 ± 9.5

FL ± SE	293.8 ± 9.0	456.6 ± 6.0	498.5 ± 3.0	279.7 ± 16.1	368.1 ± 14.5	419.6 ± 4.5

Table 3. Metazoan parasites in *Alosa alosa* (96) and *Alosa fallax* (78) in North-East Atlantic waters and connected rivers: abbreviation (abb), microhabitat in fish (MH), total prevalence (P ± SE %), mean abundance (A ± SE), and bibliographic data on salinity, infection pathway and definitive host (Kabata 1964; Chiriac and Udrescu 1973; Bray and Gibson 1980; Kennedy 1981, 2006; Gibson and Bray 1986; Køie 1993; Pasternak et al. 2000; Klimpel and Rückert 2005; Mattiucci and Nascetti 2008; Taverny and Elie 2010; Smit et al. 2014; Gérard et al. 2016)**.** BC = body cavity, DT = digestive tract, ESC = esophagus-stomach-caecum, G = gills, I = intestine, PC = pyloric caeca, S = skin; FW = fresh water, BW = brackish water, SW = salt water; Hi = intermediate host, Hp = paratenic host.

			Alosa alosa		*Alosa fallax*		Bibliographical data		
Parasite taxa	Abb	MH	P ± SE %	A ± SE	P ± SE %	A ± SE	Salinity	Infection pathway	Definitive host
Monogena									
Mazocraes alosae	*Malo*	G	99.0 ± 1.0	54.8 ± 6.5	82.1 ± 4.3	21.2 ± 4.6	FW, SW	Active (eggs laid on host gills)	*Alosa* spp.
Digenea									
Hemiurus appendiculatus	*Happ*	ESC	57.3 ± 5.0	23.4 ± 4.5	88.5 ± 3.6	30.5 ± 6.0	SW	Hi2 ingestion (copepods, chaetognaths)	Teleosts
*Parahemiurus merus**	*Pmer*	ESC	3.1 ± 1.8	0.2 ± 0.1			SW	Hi2 ingestion (copepods, chaetognaths)	Teleosts
Pronoprymna ventricosa	*Pven*	I, PC			38.5 ± 5.5	27.9 ± 6.5	FW, SW	Hi2 ingestion (amphipods)	Clupeids
Cestoda									
Eubothrium fragile	*Efra*	I, PC	1.0 ± 1.0	0.01 ± 0.01	28.2 ± 5.1	0.6 ± 0.2	SW	Hi2 ingestion (copepods)	*Alosa* spp.
Acanthocephala									
Pomphorhynchus laevis	*Plae*	I			1.3 ± 1.3	0.01 ± 0.01	FW	Hi2 ingestion (amphipods)	Teleosts
Nematoda									
Anisakis spp.	*Asim*	BC	90.6 ± 3.0	75.4 ± 3.7	42.3 ± 5.6	13.3 ± 2.6	SW	Hi ingestion (zooplankton)	Mammals
Hysterothylacium aduncum	*Hadu*	DT	61.5 ± 5.0	13.4 ± 2.1	59.0 ± 5.6	17.9 ± 3.2	SW	Hi or Hp ingestion (invertebrates, fish)**	Teleosts
Copepoda									
Clavellisa emarginata	*Cema*	G	1.0 ± 1.0	0.01 ± 0.01	5.1 ± 2.5	0.1 ± 0.1	SW	Active	*Alosa* spp.
Isopoda									
*Anilocra physodes**	*Aphy*	G	1.0 ± 1.0	0.01 ± 0.01			SW	Active	Fish
Branchioura									
*Argulus foliaceus**	*Afol*	S			1.3 ± 1.3	0.01 ± 0.01	FW, BW	Active	Fish, tadpoles
Petromyzontidae									
*Petromyzon marinus**	*Pmar*	S	1.0 ± 1.0	0.01 ± 0.01			FW, SW	Active	Vertebrates

*New records of parasites

**mainly crustaceans, chaetognaths and small fish species (Køie 1993; Klimpel and Rückert 2005)

Table 4. Molecular identification of 22 nematodes (*Anisakis simplex* s.s., *Anisakis pegreffii* and *Hysterothylacium aduncum*) found in *A. alosa* and *A. fallax* from rivers (Vire, Loire, Dordogne) and coastal/estuarine waters (North Sea, North Biscay Bay, Pertuis Charentais, Adour).

	A. alosa	*A. fallax*	Total
A. simplex s.s.	Vire (3), North Biscay Bay (1)	Loire (3), Dordogne (2), North Sea (1), Pertuis Charentais (1), Adour (1)	13
A. pegreffii		Loire (1), Pertuis Charentais (2)	3
H. aduncum	Adour (2)	North Sea (1), North Biscay Bay (1), Pertuis Charentais (1), Adour (1)	6

Table 5. Prevalence (P ± SE %) and mean abundance (A ± SE) of the parasite taxa in *Alosa alosa* (96) according to the age (immatures vs adults), the sex (males vs females), and the salinity (sea vs rivers). See abbreviations of parasite taxa in Table 3.

	Immatures (N = 15)		Adults (N = 81)		Males (N = 34)		Females (N = 47)		Sea (N = 33)		Rivers (N = 63)	
	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE
Malo	100.0 ± 0.0	27.7 ± 9.3	98.8 ± 1.2	59.8 ± 7.4	96.8 ± 3.2	54.9 ± 10.4	100.0 ± 0.0	63.5 ± 10.7	100.0 ± 0.0	48.1 ± 11.7	98.4 ± 1.6	58.2 ± 7.8
Happ	66.7 ± 12.2	22.7 ± 13.0	55.6 ± 5.5	23.5 ± 4.9	67.7 ± 8.4	32.3 ± 9.1	48.9 ± 7.3	19.1 ± 5.8	57.6 ± 8.6	17.9 ± 8.3	59.0 ± 6.3	26. 2 ± 5.4
Pmer			3.7 ± 2.1	0.2 ± 0.1			6.4 ± 3.6	0.3 ± 0.2			4.8 ± 2.7	0.3 ± 0.2
Efra	6.7 ± 6.4	0.1 ± 0.1							3.0 ± 3.0	0.03 ± 0.03		
Anis	46.7 ± 12.9	14.7 ± 6.6	98.8 ± 1.2	86.7 ± 2.7	100.0 ± 0.0	81.3 ± 4.7	97.9 ± 2.1	89.4 ± 3.4	75.8 ± 7.5	44.5 ± 6.5	98.4 ± 1.6	91.6 ± 2.7
Hadu	20.0 ± 10.3	0.9 ± 0.7	69.1 ± 5.1	15.7 ± 2.4	77.4 ± 7.5	10.7 ± 2.6	68.1 ± 6.8	20.0 ± 3.7	63.6 ± 8.4	16.9 ± 3.4	60.3 ± 6.2	11.6 ± 2.7
Cema	6.7 ± 6.4	0.1 ± 0.1							3.0 ± 3.0	0.03 ± 0.03		
Aphy	6.7 ± 6.4	0.1 ± 0.1							3.0 ± 3.0	0.03 ± 0.03		
Pmar			1.2 ± 1.2	0.01 ± 0.01			2.1 ± 2.1	0.02 ± 0.02			1.6 ± 1.6	0.02 ± 0.02

Table 6. Prevalence (P ± SE %) and mean abundance (A ± SE) of the parasite taxa in *Alosa fallax* (78) according to the age (immatures vs adults), the sex (males vs females), and the salinity (sea vs rivers). See abbreviations of parasite taxa in Table 3.

	Immatures (N = 25)		Adults (N = 53)		Males (N = 11)		Females (N = 42)		Sea (N = 55)		Rivers (N = 23)	
	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE
Malo	68.0 ± 9.3	31.2 ± 12.7	88.5 ± 4.4	16.7 ± 3.0	100.0 ± 0.0	28.5 ± 11.4	85.7 ± 5.4	13.9 ± 2.5	80.0 ± 5.3	23.0 ± 6.3	87.0 ± 7.0	16.8 ± 3.7
Happ	100.0 ± 0.0	61.3 ± 14.2	84.6 ± 5.0	16.3 ± 4.7	90.0 ± 9.5	32.4 ± 21.1	83.3 ± 5.8	12.5 ± 2.9	89.1 ± 4.2	37.0 ± 8.1	87.0 ± 7.0	15.2 ± 5.0
Pven	8.0 ± 5.4	5.6 ± 4.8	53.8 ± 6.9	39.1 ± 9.9	40.0 ± 15.5	28.0 ± 12.1	57.1 ± 7.6	41.7 ± 10.9	24.0 ± 6.0	11.4 ± 3.2	65.2 ± 9.9	67.2 ± 18.3
Efra	16.0 ± 7.3	0.8 ± 0.2	34.6 ± 6.6	0.8 ± 0.2	10.0 ± 9.5	0.1 ± 0.1	40.5 ± 7.6	0.9 ± 0.3	34.5 ± 6.4	0.7 ± 0.2	13.0 ± 7.0	0.1 ± 0.1
Plae			1.9 ± 1.9	0.02 ± 0.02			2.4 ± 2.4	0.02 ± 0.02			4.3 ± 4.3	0.04 ± 0.04
Anis	20.0 ± 8.0	7.2 ± 4.1	53.8 ± 6.9	16.5 ± 3.4	60.0 ± 15.5	28.0 ± 12.4	52.4 ± 7.7	13.8 ± 2.9	34.5 ± 6.4	13.8 ± 3.7	60.9 ± 10.2	12.2 ± 2.1
Hadu	52.0 ± 10.0	15.6 ± 5.8	61.5 ± 6.7	19.3 ± 3.8	80.0 ± 12.6	19.9 ± 7.9	57.1 ± 7.6	19.1 ± 3.8	74.5 ± 5.9	24.1 ± 4.4	21.7 ± 8.6	4.3 ± 3.1
Cema	12.0 ± 6.5	0.4 ± 0.3	1.9 ± 1.9	0.02 ± 0.02			2.4 ± 2.4	0.02 ± 0.02	7.3 ± 3.5	0.2 ± 0.1		
Afol			1.9 ± 1.9	0.04 ± 0.04			2.4 ± 2.4	0.05 ± 0.05	1.8 ± 1.8	0.04 ± 0.04		

Table 7. Component communities of metazoan parasites (prevalence P ± SE%, mean abundance (A ± SE) in *Alosa alosa* according to the sampled site. Rivers = Vire, Vilaine, and Loire; coastal and estuarine waters = North Biscay Bay and Adour. See abbreviations of parasite taxa in Table 3.

	Vire (21)		Vilaine (20)		Loire (22)		North Biscay Bay (20)		Adour (13)	
	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE
Malo	95.2 ± 4.6	42.5 ± 9.3	100.0 ± 0.0	42.5 ± 9.3	100.0 ± 0.0	99.2 ± 16.3	100.0 ± 0.0	57.3 ± 18.9	100.0 ± 0.0	33.9 ± 5.2
Happ	90.5 ± 6.4	63.9 ± 11.6	55.0 ± 11.1	12.3 ± 4.7	30.0 ± 10.2	3.0 ± 2.5	60.0 ± 11.0	27.1 ± 13.4	53.8 ± 13.8	3.8 ± 1.3
Pmer			10.0 ± 6.7	0.4 ± 0.3	5.0 ± 5.0	0.4 ± 0.4				
Efra							5.0 ± 5.0	0.05 ± 0.05		
Anis	95.2 ± 4.6	77.1 ± 7.0	100.0 ± 0.0	97.5 ± 2.5	100.0 ± 0.0	100.0 ± 0.0	60.0 ± 11.0	28.5 ± 7.9	100.0 ± 0.0	69.2 ± 7.0
Hadu	23.8 ± 9.3	1.0 ± 0.6	55.0 ± 11.1	6.8 ± 4.4	100.0 ± 0.0	26.1 ± 5.3	40.0 ± 11.0	7.5 ± 3.1	100.0 ± 0.0	31.5 ± 5.2
Cema							5.0 ± 5.0	0.05 ± 0.05		
Aphy							5.0 ± 5.0	0.05 ± 0.05		
Pmar			5.0 ± 5.0	0.05 ± 0.05						

Table 8. Component communities of metazoan parasites (prevalence P ± SE%, mean abundance A ± SE) in *Alosa fallax* according to the sampled site. Rivers = Loire and Dordogne; coastal and estuarine sites = North Sea, North Biscay Bay, Pertuis Charentais, and Adour. See abbreviations of parasite taxa in Table 3.

	Loire (8)		Dordogne (15)		North Sea (11)		North Biscay Bay (16)		Pertuis Charentais (12)		Adour (16)	
	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE	P ± SE %	A ± SE
Malo	87.5 ± 11.7	29.1 ± 7.9	86.7 ± 8.8	10.3 ± 2.8	54.5 ± 15.0	32.9 ± 24.4	100.0 ± 0.0	44.4 ± 11.9	75.0 ± 12.5	9.5 ± 3.8	81.3 ± 9.8	4.8 ± 1.0
Happ	75.0 ± 15.3	21.0 ± 14.3	93.3 ± 6.4	12.1 ± 2.2	100.0 ± 0.0	94.7 ± 28.0	81.3 ± 9.8	23.6 ± 13.4	100.0 ± 0.0	35.5 ± 10.0	81.3 ± 9.8	11.8 ± 2.5
Pven	37.5 ± 17.1	24.4 ± 15.1	80.0 ± 10.3	90.0 ± 25.4	18.2 ± 11.6	5.1 ± 4.5	12.5 ± 8.3	2.5 ± 1.7	25.0 ± 12.5	15.8 ± 9.3	50.0 ± 12.5	21.4 ± 7.3
Efra	25.0 ± 15.3	0.3 ± 0.2	6.7 ± 6.4	0.1 ± 0.1	45.5 ± 15.0	0.8 ± 0.4	6.3 ± 6.1	0.5 ± 0.5	16.7 ± 10.8	0.2 ± 0.1	68.8 ± 11.6	1.5 ± 0.5
Plae			6.7 ± 6.4	0.07 ± 0.07								
Anis	50.0 ± 17.7	10.0 ± 3.8	66.7 ± 12.2	13.3 ± 2.5	9.1 ± 8.7	1.8 ± 1.8	62.5 ± 12.1	32.5 ± 10.3	8.3 ± 8.0	4.2 ± 4.2	43.8 ± 12.4	10.6 ± 3.6
Hadu	62.5 ± 17.1	12.3 ± 8.5			72.7 ± 13.4	27.7 ± 9.5	81.3 ± 9.8	23.3 ± 8.9	41.7 ± 14.2	7.8 ± 3.6	93.8 ± 6.1	32.8 ± 7.5
Cema					27.3 ± 13.4	0.8 ± 0.6					6.3 ± 6.1	0.1 ± 0.1
Afol									8.3 ± 8.0	0.2 ± 0.2		

Table 9. Statistical comparison of the total prevalences (χ^2) and the mean abundances (*F*) of each parasite taxa common to *A. alosa* (96) and *A. fallax* (78) including the influence of sympatry. See abbreviations of the six parasite taxa in Table 3.

	Effect of Host Species							Interaction Host Species x Sympatry						
	Prevalence			Abundance				Prevalence			Abundance			
	χ^2	df	*P*	*F*	df1	df2	*P*	χ^2	df	*P*	*F*	df1	df2	*P*
Malo	17.051	1	< 0.001	15.684	1	170	< 0.001	0.674	1	0.412	2.042	1	170	0.155
Happ	21.500	1	< 0.001	0.612	1	170	0.435	0.919	1	0.338	0.014	1	170	0.906
Efra	33.150	1	< 0.001	18.922	1	170	< 0.001	0.616	1	0.432	3.899	1	170	0.050
Anis	49.087	1	< 0.001	187.947	1	170	< 0.001	8.016	1	0.005	12.648	1	170	< 0.001
Hadu	0.0003	1	0.986	2.370	1	170	0.126	0.469	1	0.493	0.116	1	170	0.733
Cema	2.565	1	0.109	1.819	1	170	0.179	2.005	1	0.157	1.885	1	170	0.172

Table 10. Statistical comparison of the total prevalences (χ^2) and the mean abundances (*F*) of each parasite taxa common to *A. alosa* (96) and *A. fallax* (78) according to the developmental stage (immatures vs adults). See abbreviations of the six parasite taxa in Table 3.

	Immatures							Adults						
	Prevalence			Abundance				Prevalence			Abundance			
	χ^2	df	*P*	*F*	df1	df2	*P*	χ^2	df	*P*	*F*	df1	df2	*P*
Malo	8.689	1	0.003	0.039	1	38	0.844	6.877	1	0.009	20.346	1	131	< 0.001
Happ	11.046	1	< 0.001	3.376	1	38	0.074	12.910	1	< 0.001	1.012	1	131	0.316
Efra	0.810	1	0.368	0.867	1	38	0.358	38.362	1	< 0.001	19.602	1	131	< 0.001
Anis	3.121	1	0.077	1.020	1	38	0.319	45.993	1	< 0.001	265.053	1	131	< 0.001
Hadu	4.212	1	0.040	3.765	1	38	0.060	0.811	1	0.368	0.675	1	131	0.413
Cema	0.312	1	0.576	0.630	1	38	0.432	1.890	1	0.169	1.564	1	131	0.213

Table 11. Metazoan parasite taxa recorded in adults of *A. alosa* in this study, in the Irish Barrow river and Waterford estuary (Doherty and McCarthy 2002), and in Western Iberian Peninsula rivers (Bao et al. 2015ab).

(P = prevalence, A = abundance, SE = standard error, SD = standard deviation).

	This study (adults)		Doherty and McCarthy 2002		Bao et al. 2015ab	
Parasite taxa	P ± SE %	A ± SE	P %	A	P %	A ± SD
Monogena						
Mazocraes alosae	98.8 ± 1.2	59.8 ± 7.4	100	83.7	88.9	7.3 ± 15.0
Digenea						
Hemiurus appendiculatus	55.6 ± 5.5	23.5 ± 4.9	41.7	27.9	72.4	24.1 ± 47.9
Parahemiurus merus	3.7 ± 2.1	0.2 ± 0.1				
Pronoprymna ventricosa			16.7	2.7		
*Diplostomum gasterostei**			16.7	0.5		
*Diplostomum spathaceum**			33.3	0.8		
Acanthocephala						
Rhadinorhynchus pristis					14.8	0.4 ± 1.3
Nematoda						
Anisakis spp.	98.8 ± 1.2	86.7 ± 2.7			96.3	171.8 ± 205.5
Hysterothylacium aduncum	69.1 ± 5.1	15.7 ± 2.4	83.3	30.9	62.3	10.6 ± 15.3
Hysterothylacium osculatum			41.7	2.8		
Crustacea						
Clavellisa emarginata			8.3	1.0		
Ceratothoa italica					11.1	0.1 ± 0.3

Unidentified copepod					11.1	0.1 ± 0.3		
Vertebrate								
Petromyzon marinus	1.2 ± 1.2	0.01 ± 0.01						

*Parasites of fish eyes were only researched by Doherty and McCarthy 2002

Table 12. Metazoan parasite taxa recorded in adults of *A. fallax* in this study, in the English Severn river and estuary (Aprahamian 1985), in the Irish Barrow river and Waterford estuary (Doherty and McCarthy 2002), and in Western Iberian Peninsula rivers (Bao et al. 2015ab).

(P = prevalence, A = abundance, SE = standard error, SD = standard deviation).

	This study (adults)		Aprahamian 1985		Doherty and McCarthy 2002		Bao et al. 2015ab	
Parasite taxa	P ± SE %	A ± SE	P %	A	P %	A	P %	A ± SD
Monogena								
Mazocraes alosae	88.5 ± 4.4	16.7 ± 3.0	78.1	10.4	44.7	48.6		
Digenea								
Hemiurus appendiculatus	84.6 ± 5.0	16.3 ± 4.7	83.1	5.5	5.5	5.6	55.9	6.5 ± 14.8
Pronoprymna ventricosa	53.8 ± 6.9	39.1 ± 9.9	96.8	numerous*	100.0	327.9		
*Diplostomum gasterostei***					1.8	2.0		
*Diplostomum spathaceum***					10.3	11.0		
Cestoda								
Eubothrium fragile	34.6 ± 6.6	0.8 ± 0.2	60.7	1.1	1.0	1.2		
Acanthocephala								
Pomphorhynchus laevis	1.9 ± 1.9	0.02 ± 0.02	9.7	0.3				
Nematoda								
Anisakis spp.	53.8 ± 6.9	16.5 ± 3.4					11.7	1.8 ± 8.8
Hysterothylacium aduncum	61.5 ± 6.7	19.3 ± 3.8	40.3	11.5	34.4	34.7	48.6	1.6 ± 3.3
Hysterothylacium osculatum					7.1	7.6		
Crustacea								

Clavellisa emarginata	1.9 ± 1.9	0.02 ± 0.02	24.5	2.3	2.6	3.9	28.6	1.1 ± 3.0
Argulus foliaceus	1.9 ± 1.9	0.04 ± 0.04						
Unidentified isopod							2.0	0.02 ± 0.1

*The exact extremely high abundance of *P. ventricosa* was such that it could not be determined (Aprahamian 1985).

**Parasites of fish eyes were only researched by Doherty and McCarthy 2002