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Parasitism, condition and reproduction of the European hake (*Merluccius merluccius*) in the NW Mediterranean Sea

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Abstract

It is well known that parameters relating to physical condition and reproduction of fish provide essential data for estimating the productivity of exploited populations, as is the case with the European hake (*Merluccius merluccius*) in the NW Mediterranean Sea. Although parasitism might affect these parameters, research in this area is very scarce and, in the case of the Mediterranean, almost non-existent. This study evaluates for the first time the potential link between parasitism, condition and reproduction of the European hake. Indicators of fish energy reserves (total lipid content in liver and gonads) and reproductive capacity (fecundity, egg quality and atresia) were evaluated, as were the prevalence and intensity of infection by metazoan parasites. The results indicate that the impact of anisakid nematodes is mostly negative and occurs mainly when hake are allocating their energy reserves to gonadal development. Although the results reveal a link between parasitism, condition and reproduction, we concluded that the NW Mediterranean hake population is in equilibrium with its metazoan parasites, which are not causing severe impairment to their physical condition or reproductive capacity.

Keywords

Merluccius merluccius; Parasites; Condition indices; Energy reserves; Fecundity; Atresia

1. Introduction

Host-parasite coevolution suggests that fish are in dynamic equilibrium with their parasites (Barret, 1986) which means parasitism is often overlooked in fish health assessment. However, it is a well-known fact that several fish parasites induce changes in host behaviour and morphology (Barber and Wright, 2006; Sasal and Thomas, 2005) and some parasitic organisms can become pathogenic and even fatal in heavy infections (Poulin, 2002; Rohde, 2005; Woo and Buchmann, 2012). Moreover, parasites can regulate host population dynamics and influence community structure (Marcogliese, 2005; Sindermann, 1987). It is also well-known that parameters relating to the condition of fish are essential for estimating the productivity of exploited populations (reviewed by Shulman and Love, 1999 and Lloret *et al.*, 2012). Although parasitism may affect life history traits such as condition, reproduction and mortality, research in this field is rather scarce and mainly focused on freshwater fish species (Tavares-Dias *et al.*, 2000; Hoffnagle *et al.*, 2006; Guidelli *et al.*, 2011; among others). Thus, there is a lack of information on the effects of parasites on the productivity of exploited marine fishes worldwide and, in particular, in the Mediterranean Sea.

The European hake (*Merluccius merluccius*, Linnaeus, 1758) (henceforth: hake) is a gadoid with a wide geographical distribution that comprises the Atlantic coast of Europe and western North Africa, the Mediterranean Sea, and the southern coast of the Black Sea. In the Western Mediterranean, hake is one of the most important target species of commercial fisheries (Oliver and Massutí, 1995). Currently, all Mediterranean stocks are considered to be highly exploited and in some areas, such as the Gulf of Lion, hake stocks might have decreased beyond safe biological limits as there is a situation of increasing overexploitation (Colloca *et al.*, 2013; Lleonart *et al.*, 2003).

Owing to its wide distribution and high commercial value, hake has been broadly studied. Early works were aimed at understanding its reproductive biology as well as making the first attempts to estimate its reproductive potential (Hickling, 1935; Tsimenidis and Papaconstantinou, 1985; Sarano, 1986; among others). Subsequently, other relevant studies (Murua *et al.*, 1998, 2006; Murua and Motos, 2006) established that hake is a batch spawner with indeterminate fecundity. Thenceforth, the number of studies on its reproduction has increased, although most of them are focused on the Atlantic population (Korta *et al.*, 2010; Mehault *et al.*, 2010; El Habouz *et al.*, 2011; among others).

In addition, most research on hake condition is based mainly on simple morphometric condition factors (Costa, 2013; Ferraton *et al.*, 2007; Giacalone *et al.*, 2010; Hidalgo *et al.*, 2008; Lloret *et al.*, 2002) and only a few have evaluated lipid content with regard to fisheries ecology (Domínguez-Petit and Saborido-Rey, 2010; Domínguez-Petit *et al.*, 2010; Lloret *et al.*, 2008) or for human nutritional purposes (Küçükgülmez *et al.*, 2008; Pérez-Villareal and Howgate, 1987).

Metazoan parasites of hake have been reported in both Mediterranean and Atlantic stocks. On the one hand, Gibson *et al.*, (2003), compilers of the Host-Parasite Database of the Natural History Museum (London, UK), gathered over fifty references on helminth parasites (monogeneans, digeneans, cestodes, nematodes and acanthocephalans) found in hake. Since then, the research on helminths in hake (mainly nematodes) has been focused on genetic studies (Mattiucci *et al.*, 2004; Farjallah *et al.*, 2008; Ceballos-Mendiola *et al.*, 2010). On the other hand, parasitic copepods of hake have also been frequently recorded (Gaglio *et al.*, 2011; Grabda and Soliman, 1975; Raibaut *et al.*, 1998; Tirard *et al.*, 1996). Despite the wide variety of parasites found in hake and their suspected negative effects, little attention has been paid to the effects of these parasites on the condition and reproduction of hake. At present,

there is no recent research available and the few published studies on the subject have always focused on a single species or taxonomic group of parasites (Guillaume *et al.*, 1985; Margolis, 1970; Ramadan *et al.*, 1981; Smith and Williams, 1967). Finally, to our knowledge, there has been no research so far into the possible relationship between parasitism and reproduction of hake.

The main goal of this study is to evaluate the links between metazoan parasites and the condition and reproduction of European hake in the NW Mediterranean Sea. The outcomes are discussed from a biological and ecological perspective, in order to provide useful ideas for improving stock assessment and management of this valuable exploited species.

2. Materials and methods

2.1. Sampling

A total of 139 females of *M. merluccius* were collected from January 2010 to March 2012 from commercial trawling and longline catches in the Gulf of Lion (NW Mediterranean Sea), between the *Cap de Creus* (Spain) and the Marseille coast (France), at depths ranging from 45 to 510 m (Fig. 1). Samples were obtained at the port of Roses (Fig. 1), which is one of the most important fishing harbours in the region. Only hake over 40 cm in length were collected to ensure that all samples were mature. The specimens were placed in individual plastic bags and transported on ice to the laboratory where they were immediately dissected. For each individual, total body length (*TL*) was measured (± 0.1 cm), and total and eviscerated body weight (*TW* and *EW*), liver weight (*LW*) and gonads weight (*GW*) were recorded (± 0.1 g). The total body lengths of the 139 fish ranged from 41.3 to 73.2 cm (mean ± *SD* = 52.0 ± 5.8) and the total body weights ranged from 539.0 to 2,959.3 g (mean ± *SD* = 1,082.3 ± 414.7). Once all the macroparasites were removed from each specimen, the whole liver and one ovary lobe were frozen at -20˚C for subsequent lipid content determination. The second ovary lobe was fixed in 4% buffered formaldehyde for histological processing.

2.2. Parasitism evaluation

2.2.1. Host examination

All samples of hake were examined for metazoan parasites. First, a naked-eye examination was performed in order to remove all visible parasites from the body surface and the buccal cavity. Second, the entire viscera were removed from the body cavity, and the gills and internal organs (heart, oesophagus, stomach, intestine, spleen, gallbladder, liver and gonads) were examined using a stereomicroscope. With regard to musculature, samples were examined from six individuals; the samples were obtained by filleting and crushing the tissue onto a transillumination platform. No metazoan parasites were found in these examinations nor were there any references in the literature to cestode or trematode larvae infecting European hake muscle. Furthermore, the prevalence of anisakid larvae in the viscera is clearly higher than in the muscle tissue (Valero *et al.*, 2006). For these reasons, it was decided to regard the number of parasites in the musculature of hake as negligible.

2.2.2. Parasite collection and identification

All macroparasites were collected and washed with a saline solution (0.8% NaCl). They were first observed alive and then fixed in permanent preparations. Monogeneans, cestodes, nematodes, acanthocephalans and copepods were preserved in 70% ethanol, whereas digeneans were fixed in Bouin's solution under slight coverslip pressure. If necessary, and depending on the taxonomic group, the specimens were cleared in lactophenol or stained with borax carmine and mounted in Canada balsam. Whenever possible, the parasites were

morphologically identified to specific level following taxonomic keys and descriptions, such as Dawes (1947) for monogeneans; Williams (1958), Gaevskaja and Aljoshkina (1995) and Bray *et al.* (2008) for digeneans; Khalil *et al.* (1994) and Kuchta *et al.* (2008) for cestodes; Petter and Maillard (1987, 1988) and Køie (2001) for nematodes; Amin (1987) and Kvach (2006) for acanthocephalans; and Scott and Scott (1913) and Kabata (1992) for copepods. Since nematode larvae belonging to the *Anisakis* genus were difficult to identify to specific level, they were provisionally assigned to two clades (*Anisakis* larvae *Type I* and *Anisakis* larvae *Type II*) on the basis of their ventriculus length and the presence or absence of a mucron (Murata *et al.*, 2011). In addition, a molecular verification on a subsample of 23 worms was performed after amplification and sequencing of a 629 bp fragment of the mitochondrial Cytochrome Oxidase 2 (*cox 2*) gene, according to the protocol described by Mattiucci *et al.* (2011).

The classification 'anisakid nematodes' included all nematodes belonging to the Anisakidae family, i.e. *Anisakis pegreffii*, *Anisakis physeteris*, *Hysterothylacium aduncum* and *Hysterothylacium fabri*, plus the anisakids in early larval stage designated as 'unidentified larvae'. In the present study, the term 'ectoparasites' is used to denote the parasite species found on the surface of the hake specimens, and 'endoparasites' to denote those species found within the body.

2.2.3. Quantitative descriptors

As described by Bush *et al.* (1997), the prevalence (*P*) was calculated as the proportion of fish infected with a particular parasite species (or taxonomic group) and the individual intensity of infection as the number of individuals of a particular species in a single infected host. The mean intensity was calculated as the average number of parasites of a particular species found in the infected hosts. The median intensity and its 95% confidence interval (*CI*) were also calculated.

2.3. Condition

For each individual, the total lipid content (% wet weight) was determined following the Soxhlet method described by Shahidi (2001), which was successfully tested for Mediterranean hake in a previous study (Lloret *et al.*, 2008). Following the procedure described in Lloret *et al.* (2008), a lipid hepatosomatic index (*LHSI*) and a lipid gonadosomatic index (*LGSI*) were calculated as $LHSI = (ABSL/EW)100$ and $LGSI = (ABSG/EW)100$, where *ABSL* and *ABSG* are the absolute lipid content in liver and gonads, respectively, which were obtained by multiplying the respective lipid contents (% wet weight) by the wet weight of either the liver or the gonads. The *LHSI* was considered as an indicator of hake condition because the liver constitutes the major organ of lipid storage in this species (Lloret *et al.*, 2008), as is the case in gadoids in general (Marshall *et al.*, 1999). The *LGSI* was used as a proxy of the energy reserves available for reproduction.

2.4. Reproduction

2.4.1. Ovarian development phase and follicular atresia

The gonadosomatic index (*GSI*), which is the relative weight of ovaries, was calculated as $GSI = (GW/EW)100$. It is assumed that *GSI* increases when spawning takes place, thus this index provides information on the fish reproductive cycle. Hake ovaries were fixed and sliced transversely in their middle area. The resulting slices were embedded in paraffin, cut into 8-10 µm sections and stained with both Mallory's trichrome and hematoxylin-eosin stains. The ovarian development phases (*regenerating*, *developing*, *spawning capable*, *actively spawning* and *regressing*) were determined following Brown-Peterson *et al.* (2011). A Chi-square test

for independence indicated no significant association between year and ovarian development phase, $\chi^2(8, n = 138) = 4.5$, $p = 0.81$. Therefore, since the proportion of females in each particular phase did not differ among the three years of sampling, the data was combined and analysed as if it were a single group of samples. The prevalence of atresia (*PA*) was defined as the proportion of females with observed α-atretic oocytes, and the relative intensity of atresia (*IA*) was calculated for each female as the number of α-atretic oocytes divided by the total number of vitellogenic oocytes (α-atretic and normal). Three different fields from different histological slides of each specimen were analysed.

2.4.2. Fecundity and egg quality

Ovaries containing oocytes in migratory nucleus or hydrated stages and without recent post-ovulatory follicles (*POFs*), i.e. the empty follicular envelopes that appear following ovulation, were selected to assess the fecundity of these females ($n = 11$). As in Murua *et al.* (2006) and Recasens *et al.* (2008), homogeneity in oocyte distribution within hake ovaries was assumed. Slices from the central area of the 11 previously-selected ovaries were weighed (± 0.1 mg). Then, the oocytes were separated using a washing process and sorted by size through several sieves (from 400 to 1,000 µm) to facilitate counting and measurement of oocytes, which was carried out using a computer-aided image analysis system (*Image-Pro® Plus 5.1*; www.mediacy.com). When oocyte size distribution followed a two-component mixture model, an algorithm of the *mixtools* package (Benaglia *et al.*, 2009) for *R* software (www.r-project.org) was applied. This statistical procedure was used to describe quantitatively the properties of the overlapping mixtures and to calculate the number of oocytes belonging to the next batch. Batch fecundity (*BF*) was defined by the number of eggs spawned per batch, and the relative batch fecundity (*BFrel*) was calculated as the value of batch fecundity per gram of eviscerated female body weight.

The dry mass of hydrated oocytes was used as an indicator of egg quality. Samples of 50 hydrated oocytes (with two replicates) were removed from nine ovaries. The sample was weighed (± 0.1 mg) after drying for 24 h at 110˚C. The mean dry weight of hydrated oocytes (*ODW*) was calculated by dividing the dry weight by the number of oocytes per replicate.

2.5. Statistical analyses

The aggregated nature of parasite distributions leads to the concentration of a high proportion of parasites in a few host individuals. As argued by Rózsa *et al.* (2000), it is useful to report the confidence interval for the median intensity of infection. For this reason, the 95% *CI* was calculated, by the BCa method with 2000 bootstrap replications, using the free *Quantitative Parasitology 3.0* software (www.zoologia.hu/qp/qp.html). This software, which was developed to manage the particularly left-biased frequency distribution of parasites, was also used to compare the prevalences (Fisher's exact test) and the median intensities (Mood's median test) for each parasite taxon (Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala and Copepoda) throughout the different seasons and ovarian development phases. Bonferroni's correction was used to counteract the problem of multiple comparisons.

Once the normality of the data was tested and rejected, several non-parametric tests were performed to assess the possible effects of parasitism on hake condition (*LHSI* and *LGSI*) and reproduction (*IA*, *BF*, *BFrel* and *ODW*). The Mann-Whitney U test was used to analyse possible differences between infected and uninfected hake specimens. The Spearman's Rank Correlation coefficient was used to verify the possible relationships between the condition and reproduction parameters and the individual intensity of the infection by parasites. Both analyses were performed for each species and taxon throughout the different ovarian development phases. In order to detect any possible synergistic effects, the correlations were

also analysed with the total number of (i) individual parasites, (ii) species, (iii) ectoparasite species, and (iv) endoparasite species. The analyses performed also took into account the site of infection of anisakid nematodes (mesenteries, liver, gonads or digestive tract, i.e. stomach and intestines). The level of statistical significance adopted was $p < 0.05$.

3. Results

3.1. Condition and reproduction

The *LHSI* presented the highest mean value in September (1.11 ± 0.66) and the lowest in August (0.39 ± 0.19), while the *LGSI* presented the highest mean value in January (0.34 ± 0.32) and the lowest in May (0.01 ± 0.00).

The *GSI* presented a clear seasonal pattern. High values were observed during the winter, with the highest monthly mean in January (5.56 ± 5.19) and another noticeable peak in July (3.09 ± 1.99). The lowest monthly mean (0.53 ± 0.13) was recorded in May. Females in different reproductive phases appeared throughout the year and were found to be spawning-capable practically all year round (Fig. 2). In addition, and as shown in Figure 3, the variations of *LHSI* and *LGSI* mean values were related to the ovarian development phase, i.e. the *LHSI* decreased with increasing *LGSI*. This opposing pattern was reported for each season except summer. Thus, it appears that the indices recorded are highly dependent on the ovarian development phase. Accordingly, the analyses of the effects of parasitism on condition and reproduction were carried out in relation to the status of gonadal maturation.

Prevalence and relative intensity of atresia were also closely related to the ovarian development phase. Females in regenerating or developing phases did not present any sign of α-atresia, while females in spawning capable (*PA* = 31.3%; *IA* = 5.94 ± 10.55%), actively spawning (*PA* = 13.6%; *IA* = 57.86 ± 36.51%) and regressing (*PA* = 60.0%; *IA* = 97.57 ± 7.28%) phases, showed different levels of α-atresia.

The batch fecundity and the relative batch fecundity were calculated for six females with oocytes in the migratory nucleus stage and for five females with hydrated oocytes. Figure 4 shows the oocyte size-frequency distributions shown by each group. A Student's t-test did not show any significant differences between the two groups regarding *BF* or *BFrel*. Thus, the results were combined and the 11 females were considered as a single group of females with oocytes in advanced stage of maturation. The *BF* ranged from 35470 to 268223 eggs (mean = 137077 ± 81919) and there was a correlation with the variables of total length (Spearman's rho; $\rho = 0.62$, $n = 11$, $p = 0.043$), eviscerated weight ($\rho = 0.62$, $n = 11$, $p = 0.043$), *LHSI* ($\rho = 0.75$, $n = 11$, $p = 0.008$) and *LGSI* ($\rho = 0.73$, $n = 11$, $p = 0.011$). Meanwhile, the *BFrel* ranged from 50 to 274 eggs·g^{-1} (eviscerated weight) (mean = 127 ± 68) and was strongly associated with *LGSI* ($\rho = 0.88$, $n = 11$, $p < 0.001$). Finally, the hydrated oocyte dry weight ranged from 0.0310 to 0.0596 mg (mean = 0.0485 ± 0.0089) and did not present significant correlations with any of the analysed variables.

3.2. Parasitism

Without exception, all of the hake specimens analysed were infected with at least one parasite species. A total of 2054 metazoan parasites belonging to nineteen species were identified (Table 1). The ectoparasites were represented by five species: one monogenean and four copepods; and the endoparasites included fourteen species: four digeneans, two cestodes, six nematodes and two acanthocephalans. Since the identification of these species is based on

adult features, parasite larvae such as metacestodes, as well as nematodes in the early larval stages, were not identified to the species level.

Nematodes species belonging to the *Anisakis* (Dujardin, 1845) genus have fairly similar morphology and therefore, identifying different species based only on morphological features is difficult. However, in this study, a differentiation between two morphotypes (*Type I* and *Type II* larvae) was possible. A subsequent molecular analysis performed on a subsample of each morphotype revealed that the *Type I* larvae were *Anisakis pegreffii* (Campana-Rouget and Biocca, 1955), and the *Type II* larvae were *Anisakis physeteris* (Baylis, 1923). Since these results were in line with the results of a detailed review on hosts and distribution of anisakid nematodes (Mattiucci and Nascetti, 2008), this outcome was inferred to the remaining *Type I* and *Type II* larvae.

Nematodes were the dominant group (P = 91.37%), followed by cestodes (79.86%), copepods (33.81%) and monogeneans (18.71%). Digeneans (5.76%) and acanthocephalans (2.88%) were detected more sporadically. The Fisher's exact tests showed no significant differences between prevalences (species and taxa) from one season to another or through the different ovarian development phases. Likewise, the Mood's median test showed no significant difference in the median intensities (species and taxa) for season or ovarian development phases. Thus, neither season nor ovarian development phase affect the parasite load in hake.

As shown in Figure 5, the prevalence of the infection by nematodes belonging to the *Anisakis* genus increased with host size (average length per size class) reaching prevalences of 100% for size classes above 55 cm. The relationship between total length and individual intensity of infection by nematodes (genus *Anisakis*, *Hysterothylacium* and *Capillaria*) was also investigated using Spearman's Rank Correlation. There was a strong positive correlation between total length and individual intensity of *Anisakis* (ρ = 0.56, n = 139, $p < 0.001$), with high intensities of infection associated with fish of greater length.

3.3. Parasite effects

The significant results ($p < 0.05$) of the Mann-Whitney U test and the Spearman's Rank Correlation are shown in Table 2. Hake in the regenerating phase that were infected with the copepods, *Clavella stellata* and *Parabrachiella insidiosa*, showed lower median values of *LGSI* and *LHSI*, respectively (Mann-Whitney U test). In addition, a lower median value of *LGSI* was also found in hake infected by *P. insidiosa* that were in spawning capable phase, although in this phase there was no correlation with the individual intensity of infection (Spearman's Rank Correlation, $p > 0.05$). In both regenerating and developing ovary phases, hake infected by the monogenean, *Anthocotyle merluccii*, showed higher values of *LHSI* and a positive correlation between *LHSI* and individual intensity of infection by this monogenean.

Hake in the developing phase that were infected with anisakid nematodes in the mesenteries displayed lower median values of *LHSI* and *LGSI* compared with uninfected specimens. But when these same parasites were found in the liver during the developing phase, only *LGSI* was significantly lower. Also in the developing phase, *LGSI* was negatively correlated to the intensity of *Anisakis pegreffii* (the most prevalent nematode), to the total number of parasite species and to the sub-category 'total number of endoparasite species'. We also found a negative correlation between *LGSI* and the intensity of *A. pegreffii* for hake in the spawning capable phase. In the same phase, there were differences in the intensity of atresia (*IA*) between hake that were infected or uninfected with anisakids in the mesenteries. Although the median value was almost the same in both groups (Table 2), the Mann-Whitney U Test revealed differences in the data distribution: the maximum value of *IA* in the uninfected group

was 0.44% and in the infected group it was 30.59%. When hake was actively spawning, the hydrated oocyte dry weight (*ODW*) of specimens infected with anisakids in the liver was higher than in uninfected hake; there was also a strong positive correlation between *ODW* and anisakids in the liver. The intensity of infection by anisakids in the digestive tract was also positively correlated with *ODW* and *LGSI*, although *LGSI* presented a negative correlation with the total number of ectoparasite species. Finally, there was a positive correlation between the intensity of infection by the cestode, *Clestobothrium crassiceps*, and *IA* for hake in regressing phase.

4. Discussion

With regard to the ectoparasites, our results indicate that their impact on the condition and reproduction of hake depends on the species of parasite concerned. Hake infected with the monogenean, *Anthocotyle merluccii*, appeared to be in better condition (i.e. they had more energy reserves in the liver) than specimens that were not infected with this parasite. One possible explanation might be that when hake are in regenerating and developing phases, they prioritize the accumulation of energy in the liver rather than allocating it to the parasitic immune response, resulting in specimens with higher energy reserves in the liver having higher intensities of infection. In contrast, the results showed that hake infected by the parasitic copepods, *Clavella stellata* and *Parabrachiella insidiosa*, had lower energy reserves in the liver and gonads than specimens that were not infected with these parasites. The adult females of these two species, which belong to the Lernaeopodidae family, are permanently anchored to the host by a strong attachment organ that can cause proliferative cell changes. In response to this infection, the hosts may allocate part of their stored energy to repairing the damage inflicted by these parasites. The results also showed a negative correlation between the number of ectoparasite species (all species together) and the energy reserves in the gonads of hake in the actively spawning phase; however, no correlation was found when each species of parasite was analysed separately. This might indicate that there was a synergetic effect of the ectoparasite species. In the literature, there are contradictory results regarding the effects of specific ectoparasites on hake condition. On the one hand, Gaglio *et al.* (2011) studied the prevalence of the copepod, *C. stellata*, in hake and found that smaller hake had more parasites than larger ones, concluding that the copepod caused little damage to the hake population due to the immunological status of the larger hake. On the other hand, Guillaume *et al.* (1985) studied the influence of the blood-sucking copepod, *Lernaeocera branchialis*, on the hake's erythrocyte constants and found different types of anaemia depending on the intensity of infection. The monogenean, *A. merluccii*, reported in this study, remains attached to the gills of their hosts and feeds on mucus and epithelial cells, while the copepod, *L. branchialis*, sucks blood on the gills of its host. Hence, the impact of ectoparasites on hake might also depend on the nutritional requirements of the parasite group it belongs to.

With regard to the endoparasites, our results indicate that their impact on hake condition and reproduction is mostly negative, whichever parasite species is considered. In the developing phase, the negative correlations between energy reserves in gonads and both the total number of species and the total number of endoparasite species indicates that there might also be a synergetic effect. During gonad development in hake, anisakid nematodes in the mesenteries appeared to affect negatively the energy reserves in the liver and, specifically, the nematode *A. pegreffii* affected negatively the energy reserves in the gonads; in addition, a high intensity of infection by *A. pegreffii* may affect the gonadal energy reserves when hake reaches the spawning capable phase. In this sense, severe damage in liver that was heavily infected with anisakid nematodes has been reported (Margolis, 1970 cited by Levsen and Berland, 2012; Ramadan *et al.*, 1981). Probably, the individual intensity of infection plays an important role

in terms of the adverse effects of parasitism. Furthermore, during the spawning capable phase, the presence of anisakid nematodes in the mesenteries was related to high values of the intensity of atresia. Such a relationship may be due to a trade-off aimed at partially compensating for some of the energy taken by the parasites. Conversely, hake in the actively spawning phase with higher intensities of anisakid nematodes in the liver and in the digestive tract have better egg quality, as shown by a higher oocyte dry weight, as well as an increase in the gonad energy reserves. We can hypothesize that this paradoxical result may be due to the fact that fish become infected when actively feeding and, therefore, hake that feed more have higher energy reserves but also a greater chance of becoming infected by nematodes. However, this result must be interpreted with caution because fish in the actively spawning phase either have hydrated oocytes ready to be released or have just released a batch and, therefore, individual energy reserves in gonads are highly variable. Finally, at the end of the individual spawning season, i.e. regressing phase, atresia intensity was also positively correlated to the intensity of infection by the cestode, *Clestobothrium crassiceps*, but it should be noted that the proportion of atretic oocytes in the regressing phase is highly variable and dependent on the particular time at which the specimen is captured. Indeed, females in regressing phase are at various different stages in the process of resorption of unreleased oocytes, i.e. recycling the energy used in gamete development and making it available, thus improving body condition.

There is a considerable amount of literature on nematodes because (i) they are the dominant group parasitizing hake and (ii) some anisakid nematodes may cause human diseases. Disregarding discrepancies that may be due to variation in methodologies, anisakid nematodes prevalences and intensities reported in our study are within the average ranges reported in previous studies (Abollo *et al.*, 2001; Mattiucci *et al.*, 2004; Valero *et al.*, 2006; Angelucci *et al.*, 2011). However, it is noteworthy that the maximum intensity of infection by anisakids in Atlantic waters is clearly higher than it is in the NW Mediterranean Sea, reaching intensities of up to 3,400 larvae in a single infected hake (Ceballos-Mendiola *et al.*, 2010) compared with a maximum of 72 larvae found in our study. In this sense, another result that should be noted is the relationship between prevalence and intensity of nematodes and the size of hake. It seems that larger hake harboured more *Anisakis* nematodes than smaller ones. By contrast, there is no similar relationship with other nematodes, not even with other genera of the Anisakidae family, such as *Hysterothylacium*. This may be related to the life cycles of nematodes. Hake are among a number of fish that can become the definitive host for *Hysterothylacium*, which will reproduce, eventually die and be expelled from the hake's body. In contrast, hake are intermediary hosts for *Anisakis* larvae. These larvae remain embedded in the tissue of the hake at the highly resistant L3 larval stage, where they wait until the hake is preyed upon (or dies and is fed upon) by the final host, such as cetaceans. Over the course of their lives, hake may accumulate more of these L3 *Anisakis* larvae, which are more difficult to eliminate than other nematodes. This would explain how more of these larvae were found in larger hake. Valero *et al.* (2006) also found higher values of *Anisakis* spp. prevalence in larger hake. These findings have important implications for human health risk assessment since *Anisakis* larvae can cause a clinical disease in humans known as anisakidosis.

Concerning hake reproduction, the results of this study indicate that Mediterranean hake has a protracted spawning season which continues practically all year round with a spawning peak in winter and another short peak in summer, which is consistent with results obtained in different Mediterranean areas (Recasens *et al.*, 2008; Al-Absawy, 2010). The joint analysis of energy reserves in liver and gonads showed a fluctuation in the amount of lipids between tissues depending on the ovarian development phase. In other words, gonads develop in detriment to the accumulated lipidic energy in the liver. This reproductive strategy, known as *capital breeding*, has been reported for other gadoids (Marshall *et al.*, 1999), but interestingly,

in summer, it seems that hake change their strategy and behave like an *income breeder*, i.e. the energy allocated to reproduction comes from concurrent feeding. It should be noted that Domínguez-Petit and Saborido-Rey (2010) demonstrated that European hake in the North Atlantic always develop the gonads when there is an energy intake surplus rather than doing so at the expense of accumulated energy. The reason for this discrepancy between the North Atlantic and the Mediterranean might be that hake is such a plastic species that it can adapt its breeding strategy to the particular biotic and abiotic factors that characterize each geographical area. The Mediterranean is a poorer sea, in terms of food, but in summer there are many more resources available since the peak of abundance of most prey populations occurs in this season (Bozzano *et al.*, 1997), thus leading to a change in the reproductive strategy of the species.

The values of fecundity are consistent with previous studies performed in the Mediterranean (Recasens *et al.*, 2008), in the North Atlantic (Korta *et al.*, 2010; Murua *et al.*, 1998, 2006) and in the eastern central Atlantic (El Habouz *et al.*, 2011). Batch fecundity increases proportionally with the size of hake, and it should be noted that correlations between batch fecundity and energy reserves, i.e. *LHSI* and *LGSI*, are still stronger than the correlations with hake size. Analogous findings reported by Domínguez-Petit and Saborido-Rey (2010) led the authors to hypothesize that larger females have higher fecundity because they have a greater capacity for obtaining energy than smaller individuals. Prevalence and relative intensity of atresia follow the expected values for species with indeterminate fecundity, such as in the hake stock of the North Atlantic (Murua and Motos, 2006). Finally, the results of the hydrated oocyte dry weight, which were used as an indicator of egg quality, showed values that were decidedly similar to an earlier study carried out on the North Atlantic population (Mehault *et al.*, 2010).

Overall, the results seem to be consistent with the hypothesis that there is a dynamic equilibrium between hosts and parasites (Barret, 1986), because potent effects of metazoan parasites on hake condition and reproduction were not detected within the infection intensities observed. Furthermore, no new parasites infecting hake, which could generate a new immune response and an eventual decline in physical condition or reproductive capacity in this species, have been found to date. In this sense, and taking into account new species interactions arising from the biological consequences of global climate and anthropogenic change, we consider it is important to continue monitoring parasitism in commercial species such as hake, as well as the possible additive or synergistic effects on hake condition and reproduction resulting from an eventual increase of the parasite load in the future.

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Table 1. Taxonomic composition, number of infected hosts, prevalence and intensity of metazoan parasites found in *Merluccius merluccius* from the Gulf of Lion (NW Mediterranean Sea). The parasite development stage, the site of infection and the ovarian development phase of hosts are also shown.

Parasite species	Stage	Site	Ovarian development phase	Infected hosts	P% (*n*=139)	Intensity		
						Min-Max	Mean ± *SD*	95% *CI*
Monogenea								
Anthocotyle merluccii (van Beneden & Hesse, 1863)	*A*	*G*	*REG, DEV, SC, AS, REGR*	26	18.71	(1-4)	1.54 ± 0.76	1.31 - 1.87
Digenea								
Aporocotyle spinosicanalis (Williams, 1958)	*A*	*H*	*AS*	1	0.72	(2-2)	2	-
Hemiurus communis (Odhner, 1905)	*A*	*S*	*AS*	1	0.72	(7-7)	7	-
Hemipera magnaprostatica (Gaevskaja & Aljoshkina, 1995)	*A*	*S*	*REG, DEV, AS*	5	3.60	(1-2)	1.20 ± 0.45	1.00 - 1.40
Lecithochirium musculus (Looss, 1907)	*A*	*S*	*DEV, SC*	2	1.44	(1-2)	1.50 ± 0.71	1.00 - 1.50
Cestoda								
Clestobothrium crassiceps (Rudolphi, 1819)	*A*	*I*	*REG, DEV, SC, AS, REGR*	107	76.98	(1-44)	4.87 ± 5.32	4.14 - 6.37
Tetraphyllidean metacestodes	*P*	*I*	*REG, DEV, SC, AS*	16	11.51	(1~100) *	13.63 ± 25.20	5.75 - 34.00
Nematoda								
Anisakis pegreffii (Campana-Rouget & Biocca, 1955)	*L3*	*I, L, M*	*REG, DEV, SC, AS, REGR*	115	82.73	(1-68)	7.78 ± 10.06	6.28 - 9.99
Anisakis physeteris (Baylis, 1923)	*L3*	*L, M*	*REG, DEV, SC, AS, REGR*	49	35.25	(1-8)	2.31 ± 1.84	1.88 - 2.92
Capillaria gracili (Bellingham, 1840)	*A*	*I*	*REG, DEV, SC, AS, REGR*	23	16.55	(1-17)	3.39 ± 4.46	2.04 - 5.83
Hysterothylacium aduncum (Rudolphi, 1802)	*L3, L4, A*	*I*	*REG, DEV, SC, AS, REGR*	24	17.27	(1-11)	2.42 ± 2.78	1.62 - 3.98
Hysterothylacium fabri (Rudolphi, 1819)	*L3, L4*	*I*	*REG, SC, AS, REGR*	6	4.32	(1-1)	1	-
Unidentified larvae	*L*	*I, S*	*REG, DEV, SC, AS, REGR*	12	8.63	(1-8)	1.58 ± 2.02	1.00 - 2.75
Acanthocephala								
Acanthocephaloides propinquus (Dujardin, 1845)	*A*	*S*	*SC, AS*	2	1.44	(1-1)	1	-
Echinorhynchus sp.	*A*	*S*	*SC*	2	1.44	(1-2)	1.50 ± 0.71	1.00 - 1.50
Copepoda								
Chondracanthus merluccii (Holten, 1802)	*A*	*B, G*	*REG, SC, REGR*	5	3.60	(1-4)	1.60 ± 1.34	1.00 - 2.20
Clavella stellata (Krøyer, 1838)	*A*	*G, Sk*	*REG, DEV, AS*	6	4.32	(1-2)	1.17 ± 0.41	1.00 - 1.33
Parabrachiella insidiosa (Heller, 1865)	*A*	*G*	*REG, DEV, SC, AS, REGR*	29	20.86	(1-6)	1.76 ± 1.15	1.45 - 2.31
Parabrachiella merluccii (Bassett-Smith, 1896)	*A*	*G*	*REG, DEV, SC*	14	10.07	(1-2)	1.21 ± 0.43	1.00 - 1.43

n, sample size. Stage: *A*, adult; *L*, early-stages larvae; *L3*, third-stage larvae; *L4*, fourth-stage larvae; *P*, plerocercoid larvae. Site: *B*, buccal cavity; *G*, gills; *H*, heart; *I*, intestines; *L*, liver; *M*, mesenteries; *S*, stomach; *Sk*, skin. Ovarian development phase of hosts: *REG*, regenerating; *DEV*, developing; *SC*, spawning capable; *AS*, actively spawning; *REGR*, regressing.

* Up to 100 metacestodes were counted from one single host, but the real intensity might be higher.

Table 2. Mann-Whitney U test used to verify the existence of differences between infected and uninfected hake and Spearman's Rank Correlation coefficient (r_s) used to evaluate possible relationships among the condition and reproduction variables and the individual intensity of parasitism. Only significant results ($p < 0.05$) are presented.

Ovarian development phase (*n*)	Parasite		Mann-Whitney U Test						Spearman's Rank Correlation		
		Variable	*n* Uninf.	*n* Inf.	*Md* Uninf.	*Md* Inf.	*U*	*p*[a]	*n*	*ρ*	*p*
Regenerating (32)	*Anthocotyle merluccii*	*LHSI*	29	3	0.480	1.134	5	0.006**	32	0.450	0.010
	Clavella stellata	*LGSI*	30	2	0.005	0.002	3	0.024	32	-0.380	0.034
	Parabrachiella insidiosa	*LHSI*	28	4	0.547	0.169	20	0.034	32	-0.370	0.035
Developing (21)	*Anthocotyle merluccii*	*LHSI*	17	4	0.704	1.284	11	0.040	21	0.441	0.045
	Anisakis pegreffii	*LGSI*	5	16	0.027	0.012	13	0.025	21	-0.509	0.018
	Anisakid nematodes in the mesenteries	*LHSI*	6	15	1.455	0.704	13	0.011	-	-	-
	Anisakid nematodes in the mesenteries	*LGSI*	6	15	0.027	0.011	19	0.045	21	-0.440	0.046
	Anisakid nematodes in the liver	*LGSI*	11	10	0.027	0.010	25	0.036	-	-	-
	Total number of species	*LGSI*	-	-	-	-	-	-	21	-0.586	0.005**
	Total number of endoparasites species	*LGSI*	-	-	-	-	-	-	21	-0.461	0.036
Spawning capable (48)	*Anisakis pegreffii*	*LGSI*	-	-	-	-	-	-	48	-0.314	0.030
	Anisakid nematodes in the mesenteries	*IA* (%)	12	36	0.000	0.000	143	0.034	-	-	-
	Parabrachiella insidiosa	*LGSI*	35	13	0.313	0.162	142	0.047	-	-	-
Actively spawning (22)	Anisakid nematodes in the liver	*ODW* (mg)	6	3	0.048	0.057	0	0.024	9	0.807	0.009**
	Anisakid nematodes in the digestive tract	*LGSI*	-	-	-	-	-	-	22	0.585	0.004**
	Anisakid nematodes in the digestive tract	*ODW* (mg)	-	-	-	-	-	-	9	0.771	0.015
	Total number of ectoparasites species	*LGSI*	-	-	-	-	-	-	22	-0.428	0.047
Regressing (15)	*Clestobothrium crassiceps*	*IA* (%)	-	-	-	-	-	-	15	0.567	0.027

n, subsample size; Uninf., uninfected fish; Inf., infected fish; *Md*, variable median. Variables: *LHSI*, lipid hepatosomatic index; *LGSI*, lipid gonadosomatic index; *IA*, intensity of atresia; *ODW*, hydrated oocyte dry weight. Anisakid nematodes encompasses: *Anisakis pegreffii*, *Anisakis physeteris*, *Hysterothylacium aduncum*, *Hysterothylacium fabri* and anisakids in early larval stage classified as unidentified larvae.

[a] Asymptotic significances (2-tailed) are displayed for Mann-Whitney U tests with sample size above 10 in all groups, otherwise, exact significances [2*(1-tailed Sig.)] are given.

** Level of statistical significance $p < 0.01$

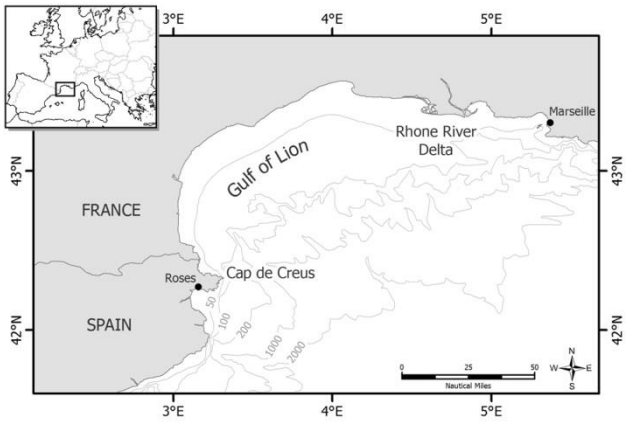

Figure 1. Map of the Gulf of Lion (NW Mediterranean), showing the port of Roses, where hake were sampled.

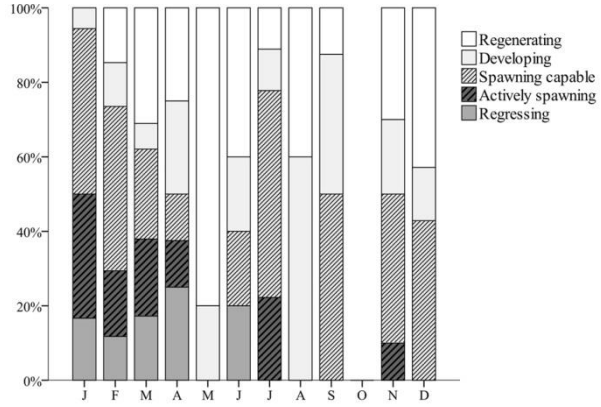

Figure 2. Monthly variations of the relative frequency of the ovarian development phases in hake (from January 2010 to March 2012).

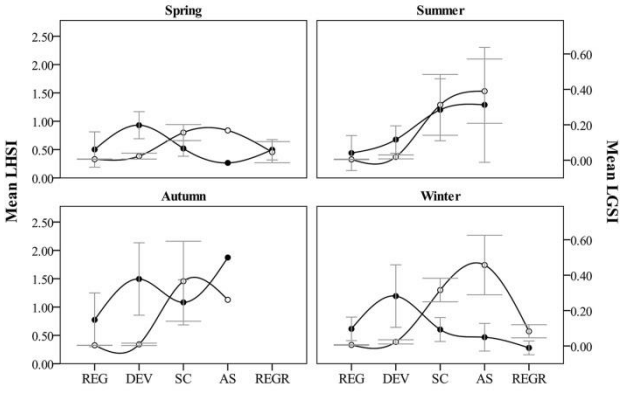

Figure 3. Seasonal mean *LHSI* (●) and *LGSI* (○) during the different ovarian development phases (REG, regenerating; DEV, developing; SC, spawning capable; AS, actively spawning; REGR, regressing). The bars represent ± standard deviations.

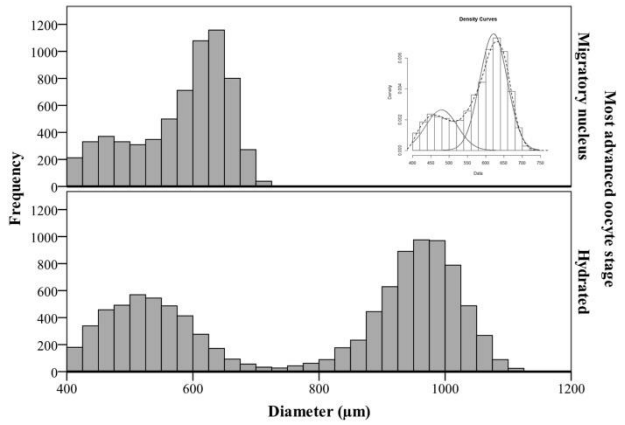

Figure 4. Oocyte size-frequency distributions (25 µm diameter class). Each distribution corresponds to an individual specimen (above, female with oocytes in migratory nucleus stage [*TL* = 46.1 cm]; below, female with hydrated oocytes [*TL* = 67.2 cm]). Since females in the migratory nucleus stage showed a two-component overlapping mixture distribution (small chart), oocytes with a 95% probability of belonging to the second component (larger diameter group) were considered as being from the next batch.

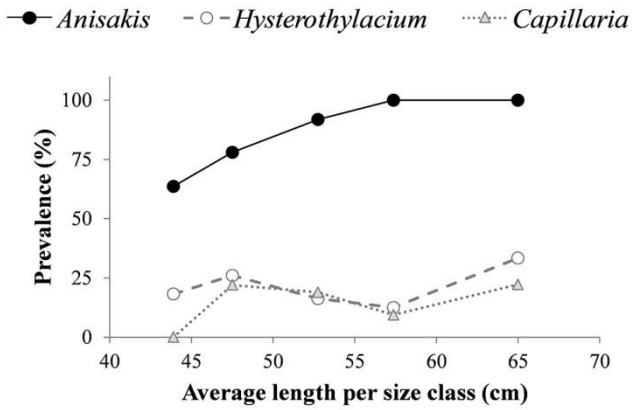

Figure 5. Prevalence of each genus of nematode in hake according to size class. The number of hosts examined per size class was: eleven specimens below 45 cm (average 43.9), fifty specimens between 45 and 50 cm (average 47.5), thirty-seven specimens between 50 and 55 cm (average 52.8), thirty-two specimens between 55 and 60 cm (average 57.4) and nine specimens above 60 cm (average 65.0).