INTRODUCTION

The Cymothoidea, one of the largest families of parasitic isopods, comprise more than 380 species (Poore & Bruce 2012, Smit et al. 2014). They parasitize a diverse array of marine, brackish, and freshwater fishes worldwide, including commercially important species (Brusca 1981, Bruce 1986, Thatcher 2006). Host specificity and host range vary among cymothoid species, ranging from 1 to >10 hosts (e.g. Yamauchi & Nagasawa 2012, Martin et al. 2015). The isopods are usually attached to a particular site on the host, which is typically consistent within species, i.e. host body surface, gills, mouth, or body cavity (Bello et al. 1997, Fogelman et al. 2009, Bottari et al. 2013), where they feed on blood, mucus, and/or tissues (Adlard & Lester 1995, Horton & Okamura 2003). Free-swimming larvae (mancae), immotile adults, and protandrous hermaphroditism characterize the cymothoid life cycle. Mancae released from the maternal marsupium seek and attach to suitable hosts (Adlard & Lester 1995, Fogelman & Grutter 2008), on which they molt several times before losing their swimming setae and becoming males. Apparently, when no female cymothoid is present on the host individual, one of the males transforms into an adult female (Adlard & Lester 1995). Cymothoids lose their swimming ability during development, and at least female cymothoids become immotile, although the timing at which this occurs is unclear (Adlard & Lester 1995).
Infection with cymothoid isopods can cause skin lesions and anemia, and it can also increase the cost of swimming for the hosts (Horton & Okamura 2003, Östlund-Nilsson et al. 2005, Binning et al. 2013). Nevertheless, many studies have found that cymothoid infection does not influence the growth and body condition of hosts (Colorni et al. 1997, Leonardos & Trilles 2003, Carrassön & Cribb 2014). In contrast, a few studies have found that cymothoid infection significantly deteriorates host condition (Gomiero et al. 2012, Sala-Bozano et al. 2012). Östlund-Nilsson et al. (2005) found no significant differences between the body condition of wild-caught cardinal fish Chaetodipterus quinquelineatus Cuvier, 1828 infected and uninfected with the cymothoid isopod Anilocra apogonae Bruce, 1987. However, when exposed to low food availability under experimental conditions, infected fish lost more weight than uninfected fish. This suggests that the negative effects of cymothoid infection on host fishes appear only when the habitat is unsuitable for the host (e.g. low food availability and high energy demand). Human activities such as coastal development and navigation have degraded the marine environment, with several degrees of intensity along the coast (Halpern et al. 2008), increasing the spatial variation in habitat suitability for marine organisms. Thus, the effect of cymothoid isopods on fish might also show spatial variation across host and parasite distributional ranges. This issue has received little attention in most fish–cymothoid systems studied, although Sala-Bozano et al. (2012) discussed possible spatial variation in cymothoid infection.

The Japanese halfbeak Hyporhamphus sajori (Temminck & Schlegel, 1846) is a pelagic fish inhabiting the coastal waters of Japan, China, and the Korean Peninsula (Nakabo 2002), which is frequently parasitized in the gill cavity by cymothoid species of the genus Mothocya (M. parvostis Bruce, 1986 and M. sajori Bruce, 1986). Despite the commercial importance of H. sajori in these countries, its total population size has recently decreased (Tsuji & Sadakata 2000). The aim of the present study was to determine the spatial variation in cymothoid prevalence and its effects on H. sajori body condition. For this purpose, Japanese halfbeak individuals were collected from 4 sites in western Japan, presenting different levels of human impact.

MATERIALS AND METHODS

Study area

Fish were sampled from 4 sites located in the Seto Inland Sea (Onomichi, Mitsuhama, and Ushimado) and in the Sea of Japan (Yoshimi), western Japan, from mid-autumn to early winter, in 2011 and 2012 (Table 1, Fig. 1). The Seto Inland Sea is a semi-enclosed coastal sea surrounded by 3 large islands...
(Honshu, Shikoku, and Kyushu), with more than 3000 small islands. This inland sea is connected to the Pacific Ocean on the east and west sides of Shikoku Island, and to the Sea of Japan via the Kanmon Straits. These 4 sites were selected to encompass the diverse marine habitats within the study area, including those influenced by human activities. The offshore areas Onomichi and Ushimado contrast with the coastal Mitsuhama and Yoshimi, with Mitsuhama being located in a ferry and fishery port of an intermediate-sized provincial city, Matsuyama, and Yoshimi in a small fishery harbor in a rural town.

Sampling and measurement of fish and their parasites

Japanese halfbeak were obtained by fishing from the shore or from a boat, except at Onomichi, where they were purchased from a fish shop (Table 1). After removing cymothoids, all fish were wet weighed to the nearest 0.01 g and their standard length (SL) was measured to the nearest 0.1 mm. The number of cymothoid individuals per fish and cymothoid total length (TL; nearest 0.01 mm), wet weight (nearest 0.001 g), and sex were recorded. Based on Bruce’s (1986) identification keys, all cymothoid individuals collected were identified as *Mothocya parvostis*, although the TL of some females was slightly larger (up to 18.71 mm) than that of *M. parvostis* (≤15 mm TL), but smaller than that of *M. sajori* (≥20 mm TL), reported by Bruce (1986). Developmental stage (i.e. mancae or not) was determined based on the morphology of the seventh pereopod. Female *M. parvostis* were classified as non-ovigerous (individuals with no visible eggs, embryos, or mancae) or ovigerous. Because cymothoid isopods are syngenetic, when only 1 ovigerous female was found in a host, it was considered that the male cohabiting with this female was accidentally lost during host sampling; in such cases, the number of individuals per fish was recorded as 2 (i.e. a female–male pair). The tiny mancae (2–4 mm TL, <0.001 g) found only on 6 fish individuals were removed from the analyses to avoid the accidental inclusion of mancae released from the female marsupium during the sampling process (Williams & Williams 1985).

Statistical analyses

Differences in parasite prevalence, number of isopods per infected fish, and number of ovigerous/non-ovigerous female cymothoids between the 4 sampling sites were tested using Fisher’s exact test followed by Benjamini and Hochberg’s correction for multiple comparisons.

To compare the effects of cymothoid infection on host body condition among the sampling sites, the concept of relative weight (Wege & Anderson 1978) was applied. First, a linear logarithmic weight–length equation was developed using the uninfected fish individuals (Log\(W = 3.2274 \log SL – 6.0268\), \(R^2 = 0.99\), \(p < 0.001\), Fig. 2A). The relative weight, \(W_r\), was then calculated for all fish according to:

\[
W_r (%) = (W/W_e) \times 100
\]

(1)

where \(W\) is the measured fish weight and \(W_e\) is the expected weight at a given body length (calculated from the weight–length equation).

Similarly, isopod body size in relation to host body size was calculated to determine the spatial variation of *M. parvostis* parasitic load. Linear logarithmic fish length–isopod length equations were developed separately for female and male isopods (female, \(\log TL_{isopod} = 0.7835 \log SL_{fish} – 0.7027\), \(R^2 = 0.69\), \(p < 10^{-5}\), Fig. 2B).

![Fig. 2. (A) Relationship between Japanese halfbeak Hypophthalmus sajori standard length and body weight; the linear regression (line) is based only on uninfected fish. (B) Relationship between Japanese halfbeak standard length and Mothocya parvostis male and female total length. Both axes are logarithmic](image-url)
0.001; male, Log TL_{isopod} = 0.61 Log SL_{fish} − 0.4314, R^2 = 0.69, p < 0.001; Fig. 2B). The relative body size of isopods, BS, was then calculated following:

$$BS_r (%) = \left( \frac{BS}{BS_e} \right) \times 100$$

where BS is the measured isopod length and BS_e is the expected isopod length at a given host length (calculated from the fish length–isopod length equation).

To examine the effects of cymothoid infection on the host, host SL and W_f were analyzed using a 2-way analysis of variance (ANOVA) considering sampling site and the number of isopods as the main factors. If the effects of cymothoid infection on the host varied spatially, such differences should be reflected in the interaction ‘site × number of isopods.’ When this interaction was significant, multiple comparisons were performed within each sampling site, using Tukey’s honestly significant difference (HSD) test. Spatial variations in the relative body size of both female and male isopods among sites were also examined using a 1-way ANOVA, considering sampling site as the main factor.

Host sex was not included as a factor in all analyses, because the gonadal index of the Japanese halfbeak is extremely low and similar between males and females during the non-reproductive season (approximately July to March in the Seto Inland Sea; Kuniyuki & Koide 1962, Yamamoto 2008), therefore having a negligible effect. Fish SL was log-transformed before analyses to improve normality. All statistical analyses were performed in R 3.1.2 (R Core Team 2014) and G*Power 3 (Faul et al. 2007).

RESULTS

Among the 161 Japanese halfbeak individuals collected from the 4 sites, 87 were parasitized by Mothocya parvostis (Table 1): 17 fish carried 1 isopod and 69 fish carried 2 isopods (including 5 fish with a single ovigerous female) in the gill cavities. Most (82.3%) of the single isopods were non-ovigerous females, 11.8% were males, and 5.9% were mancae; among the paired isopods, 98.6% were a female–male pair (including 5 single ovigerous females) and 1.4% was a male–male pair; only 1 fish was parasitized by 3 isopods (1 female and 2 males). Data on the fish parasitized by 3 isopods were pooled with that of fish parasitized by 2 isopods in the subsequent analyses. The highest parasite prevalence was found in Ushimado, followed by Yoshimi, Onomichi, and Mitsuhama, and ranged from 74.4 to 41.6% (Table 1). However, multiple comparisons showed that prevalence was only significantly different between Ushimado and Mitsuhama (p = 0.004). Based on Fisher’s exact test, the number of isopods in infected hosts and the ratio of ovigerous to non-ovigerous female isopods were not significantly different among sites (p = 0.22 and p = 0.35, respectively); the percentage of ovigerous females among all female isopods ranged from 28.6 to 51.6%.

The log-transformed SL of host fish did not differ according to the number of isopods (F_{2,149} = 2.00, power = 0.44, p = 0.14) but was different among sites (F_{3,149} = 244.79, power > 0.99, p < 0.001): fish length was significantly different between all pairs of sites (Tukey’s HSD, p < 0.001; Table 1), except between Mitsuhama and Yoshimi (p = 0.43). There was no significant interaction between sites and the number of isopods on host SL (F_{6,149} = 1.51, power = 0.61, p = 0.18). However, the effect of this interaction on host W_f was significant, indicating a spatial variation in the effect of cymothoid infection on fish condition (number of isopods, F_{2,149} = 11.88, power > 0.99, p < 0.001; site, F_{3,149} = 6.75, power = 0.98, p < 0.001; number of isopods × site, F_{6,149} = 3.20, power = 0.94, p = 0.006). Multiple comparisons at each site showed that fish body condition was only significantly affected by cymothoid infection in Mitsuhama (Fig. 3). At this site, the relative weight of infected fish was significantly lower than that of uninfected fish (Tukey’s HSD, p < 0.05) but was similar between infected individuals with different parasite loads (p = 0.90). In infected fish, W_f and SL were significantly and positively correlated (r = 0.22, p = 0.04; Fig. 4A), but this...
was not the case in uninfected fish ($r = -0.04, p = 0.77$; Fig. 4B). However, when Mitsuhama data were removed, there was no significant correlation between the relative weight and SL of infected fish ($r = -0.05$), although SL range was maintained. Thus, the relationships observed in Mitsuhama appear to be attributable to the unique characteristics of this site, rather than simply reflecting size-dependent vulnerability of the host.

Sala-Bozano et al. (2012) compared the effects of cymothoid infection on host growth and body condition between an area close to a marine protected area and an area of intense fishing pressure, and only found negative effects in the fishing area. This suggested that the energy loss caused by cymothoid infection could be readily compensated for in natural environments, by allocating extra time to feeding, but not in highly human-impacted environments, due to the increased energy demand to cope with the harsh environment and/or the limited energy intake resulting from low food availability (Östlund-Nilsson et al. 2005). In the present study, Mitsuhama was the most human-impacted area among the sampling sites. Although an evaluation of habitat quality was not conducted, human activities typically degrade fish habitat (e.g. by deteriorating water quality and reducing prey abundance). In addition, Mitsuhama is a strategic place for sea navigation. Navigation activities are known to negatively affect the abundance of certain fish species and alter their schooling behavior (Sandström et al. 2005, Sarà et al. 2007). Therefore, the Japanese halfbeak in Mitsuhama might be subjected to several stresses associated with human activities, resulting in a decrease in energy compensation against parasite infection.

On the other hand, $M.\ parvostis$ infection had no significant effects on Japanese halfbeak body condition at the other 3 sites. Although the negative effects of parasites might be too small to be detected with the sample sizes used in this study, many authors have reported similar benign effects of cymothoid infection on host fishes (Colorni et al. 1997, Leonardos & Trilles 2003, Carrassón & Cribb 2014), which seems to be closely related to the life history characteristics of cymothoids. For mature cymothoid isopods, killing their host would impose a high cost, as they are unable to swim after maturation and hence cannot find a new host (Smit et al. 2014). In such host–parasite systems, parasites might have evolved to optimize the intensity of nutritional exploitation of
their host to a degree that will not kill the host and allows the parasite to grow with the host (Jensen et al. 2006). Positive correlations between cymothoid and host body sizes have generally been found (Colon et al. 1997, Leonarzos & Trilles 2003, Carrassón & Cribb 2014), as was the case in our study. Taken together, current fish–cymothoid systems might have achieved equilibrium through the process of coevolution between parasites and hosts in their natural habitat. Serious effects of infection with native cymothoids on the growth and survival of farmed fish have provided support for this hypothesis (Sievers et al. 1996, Papapanagiotou & Trilles 2001).

The prevalence of M. parvostis on Japanese halfbeak in the present study (41.6–74.4%) was relatively high among known fish–cymothoid systems, where prevalence is typically less than 30%, e.g. approximately 14% in Paracymothoa astyanaxi Lemos de Castro, 1955 parasitizing Astyanax intermedius Eigenmann, 1908 (Gomiero et al. 2012) and up to 28% in Anilocra nemiperti Bruce, 1987 parasitizing Scolopsis bilineata (Bloch, 1793) (Roche et al. 2012). However, some studies have reported a similar high prevalence of cymothoid isopods (e.g. >40% in Leonarzos & Trilles 2003). These variations might be partly due to differences in the ecology, life history and life cycle, behavior, and population structure of the host fish, as well as to differences in cymothoid ecology and life history. However, most of these features are not known for the Japanese halfbeak–M. parvostis parasitic system (and for many other host–cymothoid systems), pointing out the need for further studies on both host and parasite fundamental ecology to elucidate the mechanisms determining parasite prevalence.

CONCLUSION

This study demonstrated that the effect of Mothocya parvostis infection on Japanese halfbeak body condition varied among sampling sites. Such spatial variation in the effects of cymothoid infections might partly explain the inconsistencies found between previous studies, which were conducted mainly at a single study site (Bello et al. 1997, Leonarzos & Trilles 2003, Carrassón & Cribb 2014). To better understand the effects of cymothoid infection, several study sites covering a variety of habitats, as well as host body size, should be included in future studies. This approach is even more important in a scenario where human activities increase the spatial heterogeneity of anthropogenic stresses. Our results also suggest that anthropogenic stresses might disrupt the host–parasite dynamics achieved through a long coevolutionary history.

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