# Health Impacts of Cymothoid Parasitic Isopods on *Symphodus tinca* and *Boops boops* Hosts in the Northwestern Mediterranean Sea at STARESO, Corsica, France

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ABSTRACT: The present study is an attempt at understanding the relationship between cymothoid parasitic isopods and the body condition of their hosts. Three species of parasitic isopod including *Anilocra physodes*, *Nerocila bivittata* and *Cymothoa oestroides* as well as two species of host fish, *Boops* boops and Symphodus *tinca,* were collected for study. A standard length to body mass relationship was established for each host species by means of a linear regression analysis. From this, the means of mass residuals for both parasitized and non-parasitized fish were analyzed to reveal that *S. tinca* hosts suffered a significantly higher health detriment than *B. boops* hosts, who showed little to no change in weight. Furthermore, roaming fish surveys designed to estimate the relative abundance of parasitized individuals within each local population of host species were conducted along three specific transects. These surveys found varying abundances of parasitized fish according to host size and location.

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## INTRODUCTION

Cymothoids (Crustacea, Isopoda) are parasitic isopods inhabiting both marine and freshwater systems. The cymothoid family is among the largest of parasitic isopod families and include at least 380 species total (Smit *et al*., 2014). They exist in all of the world’s oceans excluding polar waters, however the vast majority of cymothoid species inhabit the tropical waters of the Central Indo-Pacific. Furthermore, 35 of these species reside in the Northern Atlantic Ocean and Mediterranean Sea, the research location for this study (Smit *et al*., 2014). While cymothoids parasitize hundreds of species of fish worldwide, little is known about the specific biology and life history of many cymothoid species (Smit *et al*., 2014). These parasites associate themselves with many commercially and economically important fish around the world and could cause significant financial loss to fisheries by stunting, damaging or even killing these fish (Samn *et al*., 2014). With the recent growth of the sea-cage aquaculture practices, events of cymothoid parasitism have been shown to significantly affect cultured fish stock, and are all suspected to have been transmitted from wild fish (Horton *et al*., 2001). A better understanding of these parasites and the relationships with their hosts may help mitigate losses incurred by parasitism in aquaculture.

While cymothoid parasites may damage sea cage aquaculture, Hudson *et al.* suggests that, specialized parasite biodiversity (Hudson et al., 2006). Parasites provide a means of natural population control, however parasites do not only target fish species with potentially damaging population sizes, they can threaten endangered species as well.

Cymothoids live mainly attached to the exterior of the host fish on the skin or fins (Smit *et al.*, 2014). They primarily settle on the caudal fin, operculum, or sometimes in the mouth or gills of their host (Öktener and Trilles 2004). In this study three specific species of Cymothoidae (a specific family of parasitic isopoda) were observed to settle in three different areas of the host fish and were associated with two specific host species. First, *Anilocra physodes* was observed to parasitize *B. boops* and was found generally attached to the caudal peduncle and gill covers. Second, *Nerocila bivittata* is found on *S. tinca* most commonly on basal regions of the caudal rays. Finally, *Cymothoa oestroides* also known as the “tongue eating parasite,” was found on a *B. boops* individual and is exclusively confined to the mouth and gills of its host.

At STARESO Station de Recherches Sous Marines et Océanographiques in Calvi, Corsica, France, a number of *S. tinca* and *B. boops* individuals were observed to be carrying ectoparasitic isopods of various sizes and numbers. This study will investigate how these parasites affect the health of the host fish and that of the entire fish population by asking these questions:

1. *Do parasitic isopods affect the health of S. tinca and B. boops individuals at STARESO research station in Calvi, Corsica?*

We hypothesize that **a.)** Isopod parasites cause a negative health impact to their individual hosts. Comparisons between the relationships of body mass to standard length observed in non-parasitized and parasitized fish will be used to show the health detriment (lower unit body mass per unit body length) caused by incidences of parasitism.

We hypothesize that **b.)** Incidences of parasitism in *S. tinca* and *B. boops* will affect the hosts to differing degrees. The proportional weight loss experienced by individuals of both species, as determined by the relationships of body mass to standard body length, will be compared to assess the degree to which parasites affect their respective host species.

2. *To what degree do parasitic isopods affect local S. tin*

We hypothesize that incidences of parasitism are not frequent enough to cause significant detriment to the total population of *S. tinca* and *B. boops*. Fish population surveys will examine the prevalence of parasitized *S. tinca* and *B. boops* individuals within the total population of each species at STARESO.

## MATERIALS AND METHODS

*Species Description*

Marine parasitic isopoda (Cymothoidae) are likely a central and dominant group of crustacean parasites in fish and have been known to cause detrimental effects on fish health, growth, behavior, fertility and mortality. In this study three specific species of cymothoidae are studied; *Anilocra physodes*, *Nerocila bivittata* and *Cymothoa* *oestroides*, all three of which feed on the blood and tissue of their hosts (Öktener and Trilles 2004). Their appendages are highly specialized allowing them to securely hold the body of fish and efficiently tear the strong body muscles of host fish (Samn *et al*., 2014). Cymothoidae represent only one of the five known families of cymothoid isopoda and are exclusively parasitic on marine and freshwater fishes (Samn *et al*., 2014). This specific family of cymothoid is one of the most well known isopoda families to date, having a large number of species discovered and described before 1950 (Smit *et al*., 2014).

Cymothoidae are protandrous hermaphrodites and, starting life as male, spend a short period of their pre-host lives as free swimming *manca* (a post-larval stage). These young isopods have hatched in the marsupium of their mother and undergone several moults (pullus l and pullus ll) until they finally leave the brood pouch in search of a host (Smit *et al*., 2014). The undeveloped and sexually non-differentiated isopods are extremely fit for swimming and can survive without a host for several days by feeding on yolk stores (Smit *et al*., 2014). Once an individual has settled on a suitable host it becomes sexually differentiated (male) yet remain capable of leaving that host if necessary as long as they have not molted into the following pre-adult form. However, once an individual has reached an appropriate size and found a host they transform to female and eventually lose their ability to swim (Smit *et al*., 2014). This may be why the three species mentioned above and examined in this study were more commonly found in pairs on a single fish, one (we believe the female) being much bigger than the other. Cymothoidae species reproduce all year and females have been known to carry more than 300 eggs in her marsupium during one reproductive cycle.

In the present study, two different species of host were observed. *Symphodus tinca*, a reef associated labrid species found on rocky, algae covered reefs and seagrass meadows, has been documented to host up to 18 species of metazoan parasites (Campos and Carbonell 1994). *Boops boops* on the other hand is a demersal, semi-pelagic fish species known to host about 67 different species of metazoan parasites (Olmo *et al*., 2007) and has been suspected in some cases to transmit *C. oestroides* to cultured sea bream, *Sparus auratus,* and sea bass, *Dicentrarchus labra*x (Horton *et al.*, 2001).

*<u>Site Description</u>*

All experiments and surveys were done in Calvi, Corsica, France at STARESO research station in the months of September and October 2014. Fish specimens were collected by spear while skin diving or on SCUBA. All fish population surveys were performed on SCUBA.

Surveys were conducted between 3 to 8 meters of depth, along the interface between coastal rocky reefs and vast *Posidonia oceanica* meadows, which dominate the ocean floor, leaving little sand exposed. These coastal algae covered reef and cobble areas were central survey sites in this study, beginning at the water's edge and extending down to the substrate and *Posidonia oceanica* meadows. Several artificial reefs exist near STARESO, but only one was observed in our study; this artificial reef is a jetty made of concrete jacks located along the Northern exterior edge of the harbor.

*<u>Impact of Parasites on Host Body Condition</u>*

In order to examine the impact of cymothoid parasites on their hosts, we conducted collections on SCUBA to obtain specimens of each host species for length and weight analysis. An initial power analysis using JMP determined that at least five parasitized individuals of each species would be required to provide significant results. After collection by spear, all specimens were photographed, weighed with a digital scale, and measured using ImageJ software. Parasitized individuals were also weighed after the removal of the parasites. Individual parasites were photographed, weighed using an analytical scale, and measured using ImageJ software. Parasite specimens were stored in 70% ethanol.

For each host species, we used the relationships between fish standard length and body mass to describe the relative body condition of each specimen. Linear regression analyses of bivariate fits of body mass by standard length were performed using JMP to build standard weight models for each host species. From the standard weight regression line, we used JMP to compute the mean mass residuals of parasitized and non-parasitized (healthy) fish. The mean residuals of mass for each group describe on average how much the measured mass of each individual differed from the mass expected by the regression line. We used a one tailed t-Test analysis in JMP to determine whether the mean mass residuals of parasitized and healthy fish were significantly different (critical p-value: 0.05).

In order to compare how hosts of each species were affected by their respective parasite, we took the residuals of mass from the previously mentioned analyses and normalized them by the mass of each parasitized individual. We then compared these proportional residuals of mass for parasitized individuals across each species using a two tailed t-Test to determine if the parasites affected each host species in significantly different ways (critical p-value: 0.05).

*<u>Prevalence of Cymothoid Ectoparasitism in Local Populations</u>*

We conducted visual population surveys to assess the relative abundance of parasitized individuals in the local populations of *B. boops* and *S. tinca*. The visual nature of our surveys restricted our data collection to recording incidences of cymothoid ectoparasitism. We could not identify incidences of cymothoid endoparasitism, such as *C. oestroides* residing in the buccal cavity of hosts, without handling each individual surveyed. Our visual surveys were conducted on SCUBA along three transects at STARESO: a harbor transect, a North transect and a South transect (Figure 1).

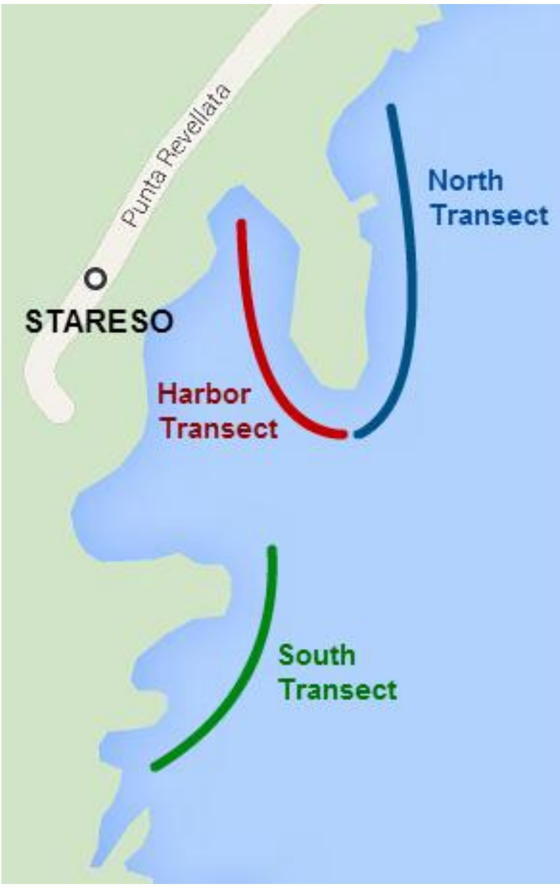


Figure 1: The harbor transect traced a 60 meter path starting from the Northern inside end of the harbor at 3 m depth, towards the mouth of the harbor, ending at an underwater anchor at 7 m depth. The Northern transect ran approximately 80 meters at 7 m depth from the mouth of the harbor, following the exterior side of the jetty from the underwater anchor towards the North, ending where the jetty met the coastline. Finally, the Southern transect ran from the Southern edge of the harbor mouth along the coastline for approximately 70 meters at 6 meter depth, ending in the second cove to the South of the harbor at a depth of 3 m. Source: Google Maps.

Roaming visual surveys divided each fish species into three size ranges[1] and counted the number of fish free of ectoparasitic cymothoids. When parasitized individuals were identified, their size range was recorded, along with information describing the number and location of parasites on the individual. Since we were looking for the relative abundance of parasitism in the population, we did not restrict the counted fish to a defined volume around the transect. We counted all of individuals of the target species that came within visual proximity to identify the presence or lack of cymothoid ectoparasites. This distance varied with visibility, however in general was about 4-6 meters.

*Impact of Parasites on Host Populations*

The data collected from the measurements of healthy specimens was used to create natural log transformed fits of body mass by standard length for each species. These functions were used, in conjunction with survey data describing the abundance of individuals in each size class, to estimate the total observed biomass for each species. After finding a rough estimate for the total observed biomass of the population, an estimate of the total biomass lost through parasitism could also be made. We multiplied the known mean residuals of mass for each parasitized individual by the total number of recorded incidences of parasitism.

## RESULTS

*Impact of Parasites on Host Body Conditions*

For *B. boops* and *S. tinca* the mean residuals of mass were compared between parasitized and non-parasitized (healthy) specimens. The mean residual of mass for healthy *B. boops* individuals was 0.541 g and the mean residual mass for parasitized individuals was -1.08g (Figure 1). The one-

[1] For *B. boops*: Small <10 cm, Medium 10-20cm, Large >20 cm. For *S. tinca*: Small <8 cm, Medium 8-15cm, Large >15 cm

tailed t-Test analysis comparing these residuals of mass found no significant difference between the two groups (t Ratio: -0.30831, DF: 10.36501, Prob < t: 0.3820). We accept the null hypothesis that incidences of parasitism cause no effect on the body condition of *B. boops* hosts, and reject the alternative hypothesis that incidences of parasitism cause a negative effect on the body condition of *B. boops*.

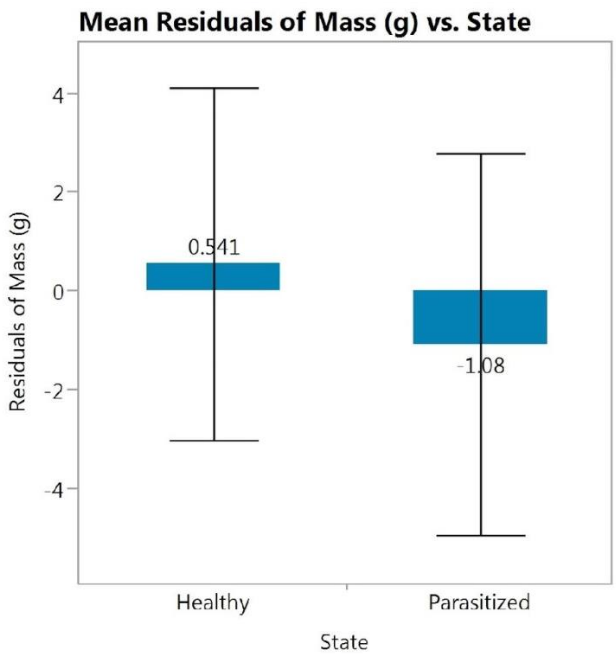


Figure 1: A bar graph representing the mean residuals of mass (g) for both the healthy and parasitized *B. boops*. The level of 0 residuals represents the linear regression model of the expected fish mass based on standard length. Each error bar is constructed using 1 standard error from the mean.

The mean residual of mass for healthy *S. tinca* individuals was 4.19 g and the mean residual of mass for parasitized individuals was -10.1g (Figure 2). The one tailed t-Test analysis comparing these residuals found a significant difference between the two groups (t Ratio: -2.39172, DF: 8.69715, Prob < t: 0.0207). We reject the null hypothesis and accept the alternative, that incidences of parasitism do cause a significant impact on the body condition of *S. tinca* individuals.

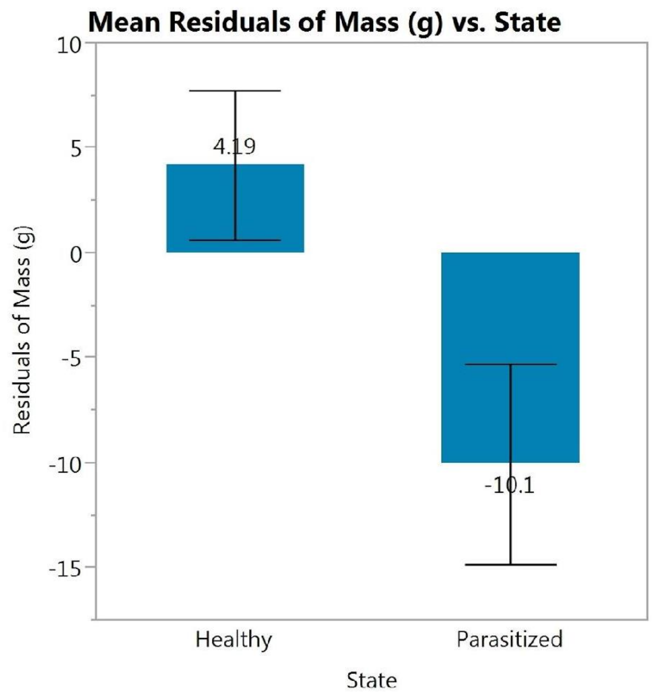


Figure 2: A bar graph representing the mean residuals of mass (g) for both the healthy and parasitized *S. tinca*. The level of 0 residuals represents the linear regression model of the expected fish mass based on standard length. Each error bar is constructed using 1 standard error from the mean.

The two tailed t-Test comparing the proportional residuals of mass for each host found no significant difference between the two species (t Ratio: -1.06896. DF 7.530962, Prob > |t|: 0.3181). We accept the null hypothesis that there is no significant difference between the proportional residuals of mass for the two species and reject the alternative hypothesis. The mean proportional residual of mass for *B. boops* was -3.35% of the host body mass and the value for *S. tinca* was 14.6% of the host body mass (Figure 3)

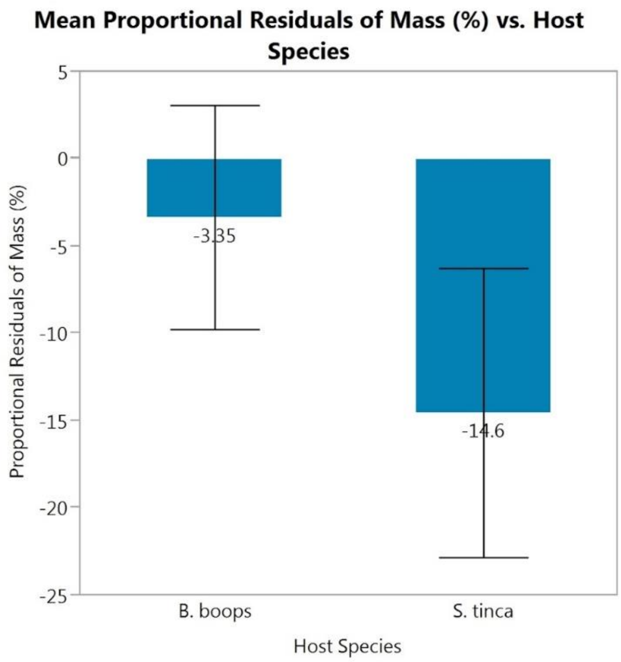


Figure 3: A bar graph representing the mean proportional residuals of mass (%) for *B. boops* and *S. tinca*. The level of 0 residuals represents the linear regression model of the expected fish mass based on standard length. Each error bar is constructed using 1 standard error from the mean.

### *Prevalence of Cymothoid Ectoparasitism in Local Populations*

Our visual population surveys found varying abundances of parasitism in subpopulations of *B. boops* and *S. tinca* hosts according to host size class and location. The overall average abundance of parasitized *B. boops* was 1.16% of the observed population (Table 1). The relative abundance of parasitized individuals in the harbor was more than double the relative abundance in the Northern transect. No individuals under 10 cm standard length were observed to carry cymothoid ectoparasites.

| | Small (<10cm) | Medium (10-20cm) | Large (>20cm) | **Total** |
|---|---|---|---|---|
| Harbor | 0.0000 | 0.0325 | 0.0351 | 0.0127 |
| North | 0.0000 | 0.0061 | 0.0000 | 0.0060 |
| **Total** | 0.0000 | 0.0224 | 0.0347 | **0.0116** |

Table 1: Relative abundance of parasitized *B. boops* observed during visual surveys, according to size class and survey location.

The overall average abundance of parasitized *S. tinca* was 1.20% of the observed population (Table 2). The relative abundance of parasitized individuals in the harbor was more than triple of that present in the Northern transect. The parasitized individuals observed in the Southern transect were all less than 4 cm in length.

| | Small (<8cm) | Medium (8-15cm) | Large (>15cm) | **Total** |
|---|---|---|---|---|
| Harbor | 0.0000 | 0.0377 | 0.0909 | 0.0217 |
| North | 0.0047 | 0.0000 | 0.0952 | 0.0062 |
| South | 0.0345 | 0.0000 | 0.0000 | 0.0185 |
| **Total** | 0.0127 | 0.0052 | 0.0423 | **0.0120** |

Table 2: Relative abundance of parasitized *S. tinca* observed during visual surveys, according to size class and survey location.

### *Impact of Parasites on Host Populations*

Based on the natural log transformed fit of body mass by standard length of *B. boops*[2] and the total population survey count (n = 3021), The total biomass of the observed *B. boops* population was approximately 114,868.6 g. The approximate biomass of *B. boops* lost to parasitism is the product of total number of incidences of parasitism (n = 35) and the mean residual of body mass of parasitized *B. boops* (-1.08 g), or about 37.8 g. This lost

[2] Expected body mass (g) = $e^{1.4849123+(0.1599715*\text{Standard Length(cm)})}$

biomass accounts for a 0.033% decrease in the total biomass of the population.

With the natural log transformed fit of body and standard length of *S. tinca*[3] and the total population survey count of 1164 individuals, the total biomass of the observed population was about 29,366.5g. The approximate biomass of *S. tinca* lost to parasitism is the product of total number of incidences of parasitism (n = 14) and the mean residual of body mass of parasitized *B. boops* (-10.1 g), or roughly 141.4g. This lost biomass accounts for a 0.48% decrease in the total biomass of the population.

## DISCUSSION

*Impact of Parasites on Host Body Condition*

In this study, three species of cymothoid parasites were found to affect two host species in distinct ways. We rejected the null hypothesis for *S. tinca,* but we failed to reject the null for *B. boops*. The parasite was less efficient in the case of the *S. tinca* having a relatively drastic effect on the health of the fish with mean residuals of mass for parasitized individuals at -10.1g. However, in the case of the *B. boops* the parasite appeared to be efficient causing little to no impact on the health of the fish. These results could highlight either the incredible resilience of *B. boops* individuals or the incredible trophic efficiency of the cymothoid parasites. This may be influenced by the fact that our weighting method left each fish ungutted. The variability between the gut contents of specimens may have concealed the true body mass differences caused by parasitism.

However, if these parasites also retard the lengths of infected fish then these effects may have been underestimated. The age of each host has not been accounted for, thus older infected fish may in fact be so severely stunted in growth that their standard length as well as body mass were reduced, concealing the true metabolic impact of the parasite. (Horton, 2001) Otolith analysis of host specimens could clarify their age and provide more meaningful comparisons with healthy fish.

On the contrary, some species of cymothoid have no noticeable negative impact on the body condition of their hosts, as the hosts may compensate by consuming more food (Östlund-Nilsson *et al.*, 2005). While these parasites may induce excess drag on hosts, increase their resting oxygen consumption and require that they spend more time foraging, they cause no negative effect upon their hosts that is observable by simple body condition measurements (Östlund-Nilsson *et al.*, 2005), as conducted in this study. Further studies examining the precise metabolics of parasitized individuals may reveal the true extent of the health impact that *A. physodes* and *C. oestroides* exert on their *B. boops*.

The comparison of proportional mass lost to parasitism in *B. boops* and *S. tinca* found no significant difference between the two host species. We will accept the null hypothesis and conclude that both *B. boops* and *S. tinca* are affected by parasitism to similar degrees. This may seem contrary to the conclusions that *B. boops* were not significantly affected by parasitism, while *S. tinca* were. This discrepancy is likely a result of the small sample sizes used for all of the mentioned analyses, where variation within each group had a greater effect.

Some species of cymothoid have significant negative effects on their hosts, while others have been observed to have no noticeable effect. Some singular species of cymothoid have been studied and found to exhibit this variety of physical effects, simply with different species of hosts (Smit *et al.*, 2014). Our findings are consistent

[3] Expected body mass (g) = $e^{1.4878166+(0.1791733*\text{Standard Length(cm)})}$

with the body of knowledge describing the wide range of health impacts that cymothoid parasites exert upon their hosts.

*Prevalence of Cymothoid Ectoparasitism in Local Populations*

Roaming fish surveys showed that the average abundance of parasitized fish observed was about 1% for both *S. tinca* and *B. boop.* However, these incidences of parasitism were not spatially homogenous, higher numbers were observed in the harbor for both host species. There was also a skewed distribution of parasitism relative to fish size. The highest percentage of parasitism was observed in large (>15cm) *S. tinca* and large (>20cm) *B. boops* individuals. However, our survey data cannot elucidate whether these spatial patterns and size associations of parasitism were the effects of mechanisms driven by the hosts or the parasites. For example, our data cannot distinguish if the higher incidence within the harbor was a result of increased host density which may have increased parasite transmission, or whether parasitized hosts from other areas migrated to seek refuge in the harbor. Our observational population data cannot inform us if the noted host-size association of the parasites is a result of preference by the cymothoids, or if the parasites cause a high rate of mortality in younger fish that conceals their affect upon this size class of hosts (Mladineo, 2003). More comprehensive population surveys could help to answer some of these questions. Larger sample sizes, obtained through larger collection operations would provide more robust data about the prevalence of cymothoid parasitism, especially endoparasitism such as *C. oestroides*, in host populations. The inclusion of a control population, unaffected by cymothoid parasitism would allow us to critically analyze the impact upon the populations near STARESO. More advanced studies on the age and local movement patterns of hosts individuals within local populations could help clarify how host population structure influences the success and resulting prevalence of cymothoid parasites in different areas.

*General Discussion*

We were surprised with the findings that *A. physodes* and *C. oestroides* did not significantly affect the body condition of *B. boops* in a negative way, while *N. bivitatta* did cause a significant negative change in the body condition of *S. tinca*. Once a parasite settles on an appropriate host, and matures into an adult male, it loses the ability to change hosts (Mladineo, 2003), so the fitness of the parasite is tied to the survival of the host. Therefore it is in the interest of the parasite to have a minimal impact on the host individual, and our studies of *B. boops* support this idea.

Our population surveys and analysis suggest that these parasites have a minimal impact on the host populations as a whole in terms of biomass. Similarly, if the population of parasites was so abundant as to cause a decrease in the size of the host population, the theoretical fitness of the parasite population would also decrease with the available host resources. The remarkable specialization of these cymothoid parasites allows them to create an ecological niche for themselves without significantly affecting the resource pool of hosts that they rely on.

## Future Studies

Multiple studies could easily branch from this work. A more extensive study with larger sample sizes and more comprehensive population surveys may provide for a more comprehensive data set. This could grant deeper insight into the host parasite relationship that exists between cymothoid parasitic isopods and the fish they live off of.

Throughout the course of this study a series of characteristics were observed in cymothoid organisms. A number of the isopods collected were brooding eggs or larvae allowing us to somewhat analyze early life behavioral habits, morphology, and swimming patterns. We observed strong phototaxis in *N. bivitatta* larvae which could indicate a tendency to swim up in the water column and could prove an interesting point of study.

We also examined host fidelity in an *Anilocra physoides* by taking it from a *B. boops* host and transplanting it to an *O. melanura*. The parasite was successfully relocated to a new host of a different species and continued to live attached to that host for several days before the trial was ended.

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