Understanding growth relationships of African cymothoid fish parasitic isopods using specimens from museum and field collections

Rachel L. Welicky, Wynand Malherbe, Kerry A. Hadfield, Nico J. Smit

ARTICLE INFO

Keywords: Cymothoa, Cinusa, Cymothoida, Host-parasite interaction

ABSTRACT

Cymothoid isopods are a diverse group of ectoparasites of fish species, and are particularly conspicuous as they are large and attach to the body surface, mouth, and gill chamber of fish hosts. These parasites transition from juvenile to male to female, and how their size changes with ontogeny and correlates with host size is not well understood. To better understand these relationships, data from field and museum collected samples of South Africa were combined to test for the associations between host and parasite length for three mouth and one gill chamber-infesting genera (Ceratoboa, Cinusa, Cymothoa, and Motherocya respectively). Generally, the number of parasites collected from 90 h of museum surveying was similar to that of seven, one-week long field collections. For two of the three mouth-infesting parasites, parasite and host size were significantly and positively correlated for males and females, but not juveniles. For gill chamber-infesting parasites, female and male parasite sizes were weakly and not significantly correlated with host size. These results provide the first morphometric data and growth relationship data for African cymothoid species and their fish hosts, and demonstrate the value and efficiency of using museum collections in ecological research.

1. Introduction

Among the most conspicuous ectoparasites of marine fishes are those of the family Cymothoidae. Cymothoids are proportionally large relative to their host, and they infest the buccal cavity, gill chamber, body surface, and body cavity of fishes (Smit et al., 2014). Parasites of this family are particularly diverse with respect to their distribution and host specificity. For example, Cymothoa sodwana Hadfield, Bruce & Smit, 2013 is found only in one location and on a single host species (Hadfield et al., 2013), and others such as Norileca indica Milne Edwards, 1840 are less host specific such that they are reported from eight fish species and have expansive distributions (Van der Wal et al., 2017). To date, taxonomists have described 43 cymothoid genera and approximately 369 species (Boyko et al., 2008 onwards), and these numbers are continuing to increase.

Our growing knowledge on the diversity and ecology of cymothoid ectoparasites is mainly limited to the adult female parasites. Cymothoids are protandric hermaphrodites, such that they mature from juvenile to male to female, and generally increase in length and mass with development (Adlard and Lester, 1994; Bunkley-Williams and Williams, 1998). Juveniles and males are substantially understudied (i.e. Williams and Bunkley-Williams, 1994) compared to adult females because they are smaller than females, making in situ observation difficult. There is also generally less known about buccal and gill infesting parasites, as they are also difficult to observe in situ as the mouth or operculum conceals them. Another challenge in observing cymothoids is the ability to locate them as their distribution appears spatially highly variable and the prevalence of infection can be very low and highly inconsistent among fish populations (Welicky and Sikkel, 2014). Hence, collecting field data on cymothoids to understand their ecology is particularly challenging with respect to time and space. There are few ecological studies on cymothoids, and often these studies take several years to collect enough data for adequate testable sample sizes, and they only focus on the adult parasites (Parker and Booth, 2013; Carrassón and Cribb, 2014; Welicky and Sikkel, 2015; Welicky et al., 2018). Such high-risk, low yield data collection has limited the rate at which we are able to learn about cymothoid ecology.

Recent studies have utilised parasites as indicators of ecosystem health (Sures et al., 2017; Vidal-Martínez and Wunderlich, 2017), and some cymothoids are quite noticeable, making them an ideal model parasite to serve as a bioindicator. Yet, before we can use these parasites as indicators of ecosystem health, we must better understand their life history and relationships with their fish hosts. Until now, such data remains sparse, mainly species-specific, and limited to localities from

https://doi.org/10.1016/j.ijppaw.2019.02.002
Received 31 October 2018; Received in revised form 1 February 2019; Accepted 3 February 2019
2213-2244/ © 2019 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).
the Caribbean, Indo Pacific, and Mediterranean (i.e. Fogelman et al., 2009; Heagney et al., 2013; Roche et al., 2013, Pawluk et al., 2015). In order to obtain information on a diversity of cymothoids, in under-studied locations, and in a time and cost efficient manner, alternative methods to field collection should be explored and undertaken. One such means of collection is to use preserved fish collections where anglers and researchers have donated fishes to museums. The possibility exists that when fish were preserved, they were infested with buccal or gill cymothoids that were subsequently trapped inside their hosts. This would then present an opportunity to gather morphometric and prevalence information on these parasites, from a wide variety of fishes collected over an expansive distribution and in a more cost and time efficient manner than field collections.

Thus the aim of this study was to understand the growth relationships between African cymothoid fish parasitic isopods and their hosts using specimens from museum and field collections. The data were examined for correlations between host and parasite length, and to determine if host size may constrain parasite size.

2. Methods

During 2009 and 2010, the South African Institute for Aquatic Biodiversity (SAIAB) marine fish collections were examined for fishes with cymothoid infections. Each fish was examined for cymothoids by visually surveying the gills and mouths of potential host species (Fig. 1). The total number of fishes surveyed was recorded. If parasites were observed, the total length of the host was recorded (in mm), and the parasite(s) was carefully extracted using forceps to not damage the host fish. Collected parasites were identified to species level, identified as juvenile, male or female, and their total length (in mm) was recorded. Parasites were generally in good condition such that their bodies were not bent or deformed. If an isopod appeared bent, it was gently straightened by pressing on the center of its body and then measured. This entire process took approximately 90 h to complete. To ensure accurate identification, species were verified using the relevant literature (Hadfield et al., 2010, 2013, 2014, 2015). Data on fish mass were not recorded as samples were preserved differently, thereby altering their masses and lacking consistency across specimens for relative comparisons. Additional specimens (n = 119) were obtained from week-long sampling trips to Tsitsikamma National Park (34°1’S, 23°52’E) in 2005, 2016, and 2017; Sodwana Bay (27°32’S, 32°41’E) in 2010, 2017 and 2018; and St. Francis Bay in 2018 (34°10’S, 24°51’E) (Table 2, Fig. 2). These collection trips formed part of other research programs and targeted fish species previously reported to host parasitic isopods. Fish were collected using rod and reel techniques and following the guidelines of the relevant ethical clearance and natural resource permits. Fish and parasite morphometric data were collected in the same manner as the preserved fish from the museum.

Descriptive statistics on the length for all fishes, and parasites subdivided by stage were calculated. To determine the efficiency of searching for parasites through museum species versus angling for parasites on fishes known to be infested, the average number and standard deviation of parasites collected per method were calculated. For museum specimens, the percent of parasitised fish per fish species observed was calculated and is herein referred to as prevalence. Prevalence of field collected specimens was not calculated because these collections were part of other projects and numbers of uninfested fish were not recorded.

The association between parasite and host length was determined using Pearson correlation coefficients for both male and female parasites (Pawluk et al., 2015). Additionally, the association between males and females from the same host were also tested with a Pearson correlation coefficient. Normality of the datasets was tested using Shapiro Wilk’s test prior to analysis, and data were not normally distributed. Accordingly, Wilcoxon rank sum tests were used to determine if attachment site is a predictor of parasite size, and if there were significant associations between attachment site and proportional parasite size by parasite life stage (male, female). The response variable, proportional parasite size, was calculated by dividing parasite size by host size, as a mechanism to control for confounding correlations between host and parasite size (as reported below), and general size/age differences among species. These tests were conducted three times. First, attachment site was divided into two levels, buccal and gill dwelling. Second, attachment site was divided into three levels, palate, tongue, and gill dwelling. Third, attachment site was divided into two levels, palate and tongue to better determine if small spatial differences within the buccal cavity influenced parasite size.

3. Results

A total of eight fish species and six parasite species were identified among the museum and field collections (Fig. 1). The fish collections from SAIAB included specimens that were collected over approximately 100 years (c.1905–2005) and from various sites. Over 1,700 fish were

Fig. 1. Isopods preserved along with their fish hosts from the South African Institute for Aquatic Biodiversity (SAIAB). A. Ceratobothia famosa Hadfield, Bruce & Smit, 2014 in the mouth of Diplodus capensis Smith, (1844); B. Mothocya affinis Hadfield, Bruce & Smit, 2015 in the gills of Hyporhamphus affinis (Günther, 1866); C. Cymothoa sodwana Hadfield, Bruce & Smit, 2013 in the mouth of Trachinotus bala (Shaw, 1803).
examined from the museum collections including two species that were not caught in the field (Table 1). These two species were Ceratothoa africanae Hadfield, Bruce & Smit, 2014 and Ceratothoa carinata (Bianconi, 1869). Parasite prevalence in the various hosts ranged from 3.6 to 17.4%. Museum collection trips generally provided more data on juvenile parasites and fishes than field collection trips (Fig. 2). For most fish species, the average number of parasites collected over 3–4 field trips was similar to that observed in one museum trip (Fig. 2).

A large range in fish and parasite size by species was observed for most fishes. This was most apparent for the Cape white seabream, Diplodus capensis (Smith, 1844) and the white musselcracker, Sparadon durbanensis (Castelnau, 1861). Only two fishes were observed rarely infested which included Spondylisoma emarginatum (Valenciennes, 1830) and Selar crumenophthalmus (Bloch, 1793). For parasites, females were consistently larger than male conspecifics, ranging from 1.5 to 3.1 times the size of males (Table 2). With the exception of Ceratothoa carinata, juvenile parasites were on average about one-third to one-half the length of males (Table 2). Juvenile parasites were rarely observed on fish that were parasitised with adult parasites.

For all buccal male and female parasites, except male Cymothoa sodwana, there were significant relationships between host and parasite size (Fig. 3). The strengths of these relationships were weak except for Ceratothoa famosa Hadfield, Bruce & Smit, 2014 (Fig. 3A and B). For juvenile buccal parasites (Fig. 4), only C. famosa demonstrated a significant relationship with host size, but this relationship was weak (Fig. 4B). For gill chamber dwelling female and male Mothocya affinis Hadfield, Bruce & Smit, 2015, there were weak and non-significant associations with host size (Fig. 3G and H). The results of the male–female length relationships indicated a significant positive correlation for C. famosa (R² = 0.89; p = 0.0001), C. sodwana (R² = 0.44; p = 0.0002), Cinsusa tetrodonitis Schioedte & Meinert, 1884 (R² = 0.46; p < 0.0001) and M. affinis (R² = 0.67; p = 0.001).

Attachment site significantly influenced proportional parasite size for females and males in our analyses when comparing buccal and gill attachments, and palate, tongue, and gill attachments. Interestingly, there were significant proportional size differences between female parasites that infest locations that are within the same cavity, but this was not the case for the males (Table 3). Accordingly, palate-dwelling female parasites (Cinsusa tetrodonitis) were proportionally larger than tongue-dwelling parasites (Ceratothoa africanae, C. carinata, C. famosa

**Table 1**
The total number of fishes searched at the South African Institute for Aquatic Biodiversity (SAIAB), including the prevalence of infection for each species.

| Year | Fish species           | Total fish | No. Fish infested | Prevalence (%) | Isopod species |
|------|------------------------|------------|-------------------|----------------|----------------|
| 2009 | Amblyrhynchotes honckenii | 226        | 16                | 7.1            | Cinusa tetrodonitis |
|      | Diplodus capensis       | 1004       | 84                | 8.4            | Ceratothoa famosa |
|      | Sparadon durbanensis    | 100        | 12                | 12.0           | Ceratothoa famosa |
|      | Spondylisoma emarginatum | 68         | 4                 | 5.9            | Ceratothoa africanae |
|      | Diplodus hottentotus    | 366        | 28                | 7.7            | Ceratothoa famosa |
|      | Hyporhamphus affinis    | 112        | 4                 | 3.6            | Mothocya affinis |
|      | Selar crumenophthalmus  | 39         | 3                 | 7.7            | Ceratothoa carinata |
|      | Trachinotus bolea       | 23         | 4                 | 17.4           | Cymothoa sodwana |

*Fig. 2.* The total number, mean number and standard deviation of parasites collected from the South African Institute for Aquatic Biodiversity and fieldwork, respectively. J = juvenile, M = male, F = female. Attachment type indicated as: B = buccal, T = tongue, P = palate, G = gill.
and *Cymothoa sodwana*), and no significant differences were observed between palate- and tongue-dwelling males (Table 3).

### 4. Discussion

The prevalence of South African cymothoids from the SAIAB museum was generally smaller than what is reported for other cymothoid taxa from sampling localities around the world. Saia-Bozano et al. (2012) reported prevalence of 47% and 30% for *Ceratotthoa italica* Schioedte & Meñiert, 1883 in the Turrhenian Sea and Balearic Sea, respectively, whereas prevalence of *C. famosa* from all South African fish hosts was only 9%. Moreover, Chávez-López et al. (2005) reported a 46.3% prevalence of gill-dwelling *Eithusa alvaradoensis* Rocha-Ramírez, Chávez-López & Bruce, 2005 off the continental shelf in Mexico. This prevalence is ~13 times higher than the prevalence of gill-dwelling, *M. affinis*. Only *C. sodwana*, had similar prevalence (17.4%) to another species of *Cymothoa* (19%) (Cook and Munguia, 2015). The fact that the prevalence of South African cymothoids was smaller than the aforementioned studies, but we examined more samples representing a longer time frame, highlights that natural prevalence of cymothoids from South Africa is likely naturally smaller. It is plausible that prevalence is associated with environmental conditions and climate, and/or species differences, but the mechanisms that influence prevalence are not well-understood, and further studies are needed to address these unknowns.

The findings of the correlation analyses support previously documented patterns of adult parasite length being positively correlated with host length, and that females are typically twice the size of males (Alvarez and Flores, 1997; Cook and Munguia, 2015; Pawluk et al., 2015). When examining the morphometric data and correlation analyses, it appears that host size constrains parasite size (Tsai et al., 2001; Pawluk et al., 2015). This is likely an artefact of attachment site, and size constraint becomes seemingly more evident with parasite ontogenetic development as the parasites grow larger. For juveniles, no strong significant relationships with host size were observed. This may reflect that juveniles are relatively small compared to the area in which they infest, such that their growth may not be limited by the size of the area that they infest. For adult parasites, there were stronger positive and significant relationships with host size for buccal parasites compared to gill parasites. This likely reflects that the space provided by the buccal cavity is relatively larger than that of the gill chamber for the fishes surveyed, thereby providing more space for buccal parasites to grow.

The findings of the correlative analyses, and the explanations thereof, are consistent with the findings when host size is controlled for and proportional parasite size is examined for associations with attachment site and parasite gender. Overall, buccal parasites were significantly larger than gill parasites. Palate-infesting females were larger than tongue-infesting females, but there was no difference in size between palate- and tongue-infesting males. The palate likely provides more space to grow than the glossobyal, and the amount of available space to grow decreases as the parasite develops from male to female. Accordingly, males are not space-limited like females. Juveniles also appear to not be space-limited, or demonstrate preference for attachment site, particularly when a superinfestation occurs (Williams and Bunkley-Williams, 1994). To better understand these fine scale patterns and differences, morphological analyses should be paired with geometric and phylogenetic analyses in the future.

The most applied outcome of this research is that we have demonstrated that the use of museum collections to obtain ecological data on rare and difficult to collect species is highly efficient compared to field data collection. We note that SAIAB collections consisted primarily of juvenile fish and parasites. While in situ research along the coasts of South Africa could likely collect the same organisms, such research typically occurs over a much longer period of time because permit regulations restrict annual numbers of fishes that can be collected, and some collection trips are less successful than others due to unpredictable turbidity, roughness, and site accessibility conditions. Museum collections that have specimens catalogued with their locality data are also particularly useful for determining the broad regions in which field collections are likely to be most successful. Accordingly, museum collections provide a more time efficient and pragmatic approach than field collections, and this approach should be considered when applicable, particularly for difficult to study organisms.

### Acknowledgements

We thank the South African Institute of Aquatic Biodiversity for their assistance in this research, particularly collection manager Roger Bills, for allowing us access to the fish collections. RL Welicky received financial support from the Claude Leon Foundation for this research. Financial assistance of South Africa’s National Research Foundation (NRF) towards this research is hereby acknowledged (NRF project IFR170210222411 grant 109352, NJ Smit, PI). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF. The Department of Environmental Affairs, iSimangaliso Wetland Park, and KZN Wildlife are thanked for research support.

### Table 2

Infected fish and parasite sizes (mean ± 1s.d. and in mm). Sample sizes denoted parenthetically are and the sum of field and museum collected specimens.

| Host-Parasite Asemble | Host Length | Parasite Life Stage | Parasite Length |
|----------------------|-------------|---------------------|-----------------|
| *Amblyrhynchotes homuncnii – Cirinus tetrodonis* | 143.4 ± 36.5 (82) | Juvenile (7) | 6.0 ± 1.3 |
| *Diplodus hottenstotus – Ceratotthoa famosa* | 72.5 ± 35.7 (23) | Male (36) | 12.5 ± 2.7 |
| *Diplodus capensis – Ceratotthoa famosa* | 66.9 ± 54.7 (88) | Female (39) | 19.2 ± 3.4 |
| *Sparadon darbanensis – Ceratotthoa famosa* | 140.6 ± 115.4 (19) | Juvenile (19) | 4.8 ± 1.1 |
| *Spondylosoma emarginatum – Ceratotthoa africanae* | 17.3 ± 2.5 (5) | Female (7) | 19.7 ± 8.7 |
| *Salar crumenophthalmus – Ceratotthoa carinata* | 21.6 ± 3.0 (8) | Male (15) | 10.0 ± 3.4 |
| *Hyporhamphus affinis – Mithycya affinis* | 285.7 ± 40.8 (39) | Female (7) | 19.7 ± 8.7 |
| *Trachinotus bota – Cymothoa sodwana* | 249.8 ± 51.6 (57) | Female (28) | 23.3 ± 4.7 |

...
Fig. 3. The relationship between cymothoid size and host size for female and male parasites by parasite species.
permits RES2010/52, RES2016/30, RES2017/35, RES2018/35. The South African National Parks (SANPARKS) is thanked for research permit numbers MALH-K-2016-005 and permitted work titled, “Biodiversity and systematics of marine isopods from southern Africa”. This is contribution number 272 from the NWU-Water Research Group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jppaw.2019.02.002.

References

Adlard, R.D., Lester, R.J.G., 1994. Dynamics of the interaction between the parasitic isopod, Anilocra pomacentri, and the coral reef fish, Chromis multilineata. Parasitology 109, 311–324.

Alvarez, F., Flores, M., 1997. Cymothoa exigua (Isopoda: Cymothoidae) parasitizing the gorgonian Lophogorgia mutica (Linnaeus, 1776) from Cabo de Gata, east coast of Spain. Mar. Biodivers. 7, 21–25.

Boyko, C.B., Bruce, N.L., Hadfield, K.A., Merrin, K.L., Ota, Y., Poore, G.C.B., Taiti, S., Schotte, M., Wilson, G.D.F. (Eds.), 2008. World Marine, Freshwater and Terrestrial Vectors 10, 65.

Bunkley-Williams, L., Williams, E.H., 1998. Isopods associated with fi shes: a synopsis and corrections. J. Parasitol. 84, 893–896.

Carrascón, M., Cribb, T.H., 2014. Benign effect of the fish parasitic isopod Ceratophya cf. imbricata on Selenotoca multifasciata (Scaphidaphagidae) from Australia. Dis. Aquat. Org. 110, 173–180.

Chávez-López, R., Rocha-Ramírez, A., Wetzer, R., 2005. Elithus alfaroventrosus Rocha-Ramírez, Chavez-Lopez & Wetzer 2005 (Isopoda, Cymothoidae) parasitizing the in-shore lizardfish, Synodus poecilus (Linnæus, 1766) on the continental shelf off central Veracruz, Mexico. Crustaceana (Leiden) 78, 865–872.

Cook, C., Munguia, P., 2015. Sex change and morphological transitions in a marine ectoparasite. Mar. Ecol. 36, 337–346.

Fogelman, R.M., Kuris, A.M., Grutter, A.S., 2009. Parasitic castration of a vertebrate: effect of the cymothoid isopod, Anilocra apogonae, on the five-lined cardinalfish, Cheilodipterus quinquelineatus. Int. J. Parasitol. 39, 577–583.

Hadfield, K.A., Bruce, N.L., Smith, N.J., 2010. Redescription of the monotypic genus Cymothoa Skeoch and Meinert, 1848 (Isopoda, Cymothoidae), a buccal-cavity isopod from South Africa. Zootaxa 2437, 51–68.

Hadfield, K.A., Bruce, N.L., Smith, N.J., 2013. Review of the fish-parasitic genus Cymothoa Fabricius, 1793 (Isopoda, Cymothoidae, Crustacea) from the southwestern Indian Ocean, including a new species from South Africa. Zootaxa 3640, 152–176.

Heagney, E.C., Gillanders, B.M., Suthers, I.M., 2013. The effect of parasitism by a blood-feeding isopod on the otolith chemistry of host fi sh. Mar. Freshw. Res. 64, 10–19.

Parker, D., Booth, A.J., 2013. The tongue-replacing isopod Cymothoa borbonica reduces the growth of largespotted pompano Trachinotus bowditchi. Mar. Biol. 160, 2943–2950.

Roche, D.G., Strong, L.E., Binning, S.A., 2013. Prevalence of the parasitic cymothoid isopod Anilocra nemperi on its host at Lizard Island, Great Barrier Reef. Aust. J. Zool. 60, 330–333.

Sala-Bozano, M., van Oosterhout, C., Mariani, S., 2012. Impact of a mouth parasite in a shore fi sh: a synopsis and corrections. J. Parasitol. 84, 893–896.

Van der Wal, S., Smit, N.J., Hadfield, K.A., 2010. Redescription of the monotypic genus Anilocra Haedel, K.A., Bruce, N.L., Merrin, K.L., Ota, Y., Poore, G.C.B., Taiti, S., Schotte, M., Wilson, G.D.F. (Eds.), 2008. World Marine, Freshwater and Terrestrial Vectors 10, 65.

Tsai, M.L., Li, J.J., Dai, C.F., 2001. How host size may constrain the evolution of parasite body size and clutch size. The parasitic isopod Varicorhinus bacbatulus (Dana, 1852 (Crustacea, Isopoda, Cymothoidae) from South Africa, including the description of a new species. Zootaxa 2437, 51–68.

Welicky, R.L., Sikkel, P.C., 2014. Variation in occurrence of the cymothoid isopod, Cymothoa exigua (Isopoda: Cymothoidae), a buccal-cavity isopod from Schioedte and Meinert, 1884 (Isopoda, Cymothoidae), a buccal-cavity isopod from Schiff, 1820 (Pisces: Lutjanidae) in Manzanillo, Colima, Mexico. Rev. Biol. Trop. 44, 391–394.