

Parasitic isopods (*Gnathia* sp.) reduce haematocrit in captive blackeye thicklip (Labridae) on the Great Barrier Reef

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(Received 1 June 2004, Accepted 22 November 2004)

Captive *Hemigymnus melapterus* exposed to large numbers of cultured juvenile parasitic isopods (*Gnathia* sp.) had significantly lower haematocrit (median 27.62% \pm 5.83% inter-quartile range) than uninfected, control fish (median 32.73% \pm 4.90%). This study is the first to show that juvenile *Gnathia* sp. reduce total blood volume in *H. melapterus*. The low haematocrit in infected fish was most likely due to plasma replacing erythrocytes lost as a result of isopods feeding on fish blood.

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Key words: *Gnathia*; haematocrit; *Hemigymnus melapterus*; Isopoda; Labridae.

Juvenile gnathiid isopods are one of the most common ectoparasitic crustaceans on the Great Barrier Reef (GBR) (Grutter, 1994; Grutter & Poulin, 1998) and occur on fishes worldwide (Cohen & Poore, 1994) yet their effects on host physiological variables are little known. Gnathiids attach to the skin and gills with piercing and tearing mouthparts, and use a muscular oesophagus and grooved paragnath to feed on blood (Monod, 1926; Davies, 1981). In elasmobranchs, gnathiid infections have caused a loss of gill epithelia (Heupel & Bennett, 1999), inflammation, and epithelial and smooth muscle disruption (Honma & Chiba, 1991; Honma *et al.*, 1991). The only quantitative study of gnathiids affecting teleost physiology found that gnathiid load did not correlate with stress hormone levels in wild blackeye thicklip *Hemigymnus melapterus* (Bloch) (Grutter & Pankhurst, 2000). Gnathiids can be highly pathogenic, even fatal to teleosts at high densities (Paperna & Por, 1977; Mugridge & Stallybrass, 1983). Mugridge & Stallybrass (1983) found *c.* 100 gnathiids on dead eel *Anguilla anguilla* (L.), but it is not clear if death was due to blood feeding, stress or subsequent bacterial infection. The present study investigated the effects of *Gnathia* sp. infections on host blood erythrocyte volume (haematocrit).

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To quantify the effects that high densities of juvenile *Gnathia* sp. have on teleost haematocrit, individual ectoparasite-free *H. melapterus* were exposed to one of two treatments: exposure to infection by *Gnathia* sp. or no exposure (control). The study took place at Lizard Island Research Station, GBR, Australia (14°40' S; 145°28' E) where isopods have been cultured since 2002 (Grutter, 2003). *Hemigymnus melapterus* (mean 17.1 cm, total length, L_T) were collected from 21 to 31 May 2003 by a snorkeller using hand and barrier nets similar to Grutter (1994). Existing ectoparasite infections were removed in a 3 min freshwater bath followed by a 0.01 g l⁻¹ praziquantal (dissolved in ethanol) bath (ICN Biomedicals, Irvine, CA, U.S.A.) for 2 h on the day of capture. These ectoparasite-free fish were kept in groups of two to six individuals in flow-through 1000 l tanks and acclimated for 2 to 7 days before use. Ectoparasite-free *H. melapterus* were randomly assigned to either the isopod culture or an identical oval plastic tub without isopods (215 × 150 × 50 cm). Fish were confined for 24 h in rectangular plastic baskets (60 × 40 × 40 cm with 1 cm mesh) covered with shade cloth, then removed for blood sampling and replaced with new ectoparasite-free fish. Confinement allowed quick re-capture to avoid losing isopods due to handling (Grutter, 1995). Fish were removed from the baskets with a soft-meshed hand-net, and placed into moist re-sealable plastic bags. Bagged fish were laid on their sides, a cloth was placed over their eyes, and blood was taken by caudal venipuncture through the plastic bag. This minimized stress, which can increase haematocrit (Turner *et al.*, 1983), facilitated taking blood, and minimized loss of isopods attached to the fish. The time taken to approach the tank, capture fish and remove blood was 63–434 s (mean ± s.e. 154 ± 17 s). After the blood sampling, fish were measured, removed from the plastic bags and placed in an aerated bucket with sea water for 2 h, followed by 3 min in fresh water. The plastic bag contents, sea water and bath water were filtered through a 62 µm sieve to retrieve isopods, which were fixed in 10% formalin in sea water. Isopods were counted, measured and categorized for presence or absence of a blood meal. Haematocrit was calculated using whole blood in heparinized haematocrit tubes and spun for 5 min at 9120g in a microhaematocrit centrifuge.

All data were examined for normality and equal variances before analysis. One outlier control fish with low haematocrit (20.21%) died shortly after confinement and was excluded from analysis. Differences in haematocrit between treatments were analysed with a Wilcoxon rank-sums test, using critical values of the χ^2 distribution. Differences between mean time to take blood and mean fish L_T between treatments were tested using one-way ANOVA. All correlations were tested for significance using two-tailed critical values of the simple correlation coefficient r .

Haematocrits from fish exposed to *Gnathia* sp. were significantly lower than those of control fish (d.f. = 1, $P = 0.019$, Fig. 1). Fish L_T did not vary between treatments (ANOVA, $n = 23$, $P = 0.247$) nor was haematocrit correlated with time to take blood (ANOVA, $n = 23$, $P = 0.404$), therefore, these are unlikely explanations for the differences in haematocrit between treatments.

Ectoparasitic crustaceans can increase (Bowers *et al.*, 2000) or reduce haematocrit in fish depending on the severity of infection. If parasites simply act as a stressor, haematocrit can increase by splenic release of stored blood cells, by

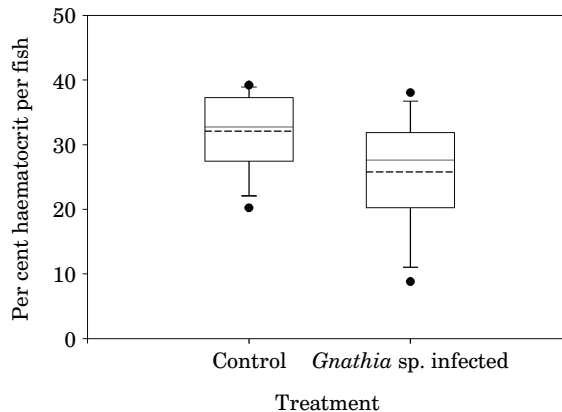


FIG. 1. Box and whisker plots of median per cent haematocrit from *Hemigynus melapterus* exposed and not exposed (control) to *Gnathia* sp. Twenty-five and 75% quartiles are displayed as boxes around the medians (—) and 10 and 90% quartiles are shown as error bars. ---, means; ●, minimum and maximum values for each treatment.

plasma loss or by erythrocytic swelling (Pearson & Stevens, 1991; Fänge, 1992). Sufficiently large or numerous ectoparasites can reduce haematocrit through blood feeding (Romestand & Trilles, 1977; Nair & Nair, 1983; Grimnes & Jakobsen, 1996; Horton & Okamura, 2003; Wagner & McKinley, 2004) or *via* osmoregulatory failure caused by exposed lesions (Grimnes & Jakobsen, 1996; Bjørn & Finstad, 1997). Lesions were not observed on infected fish, hence the results from this study are consistent with haematocrit reduction caused by parasites feeding on the blood of fishes.

The level of haematocrit where tropical wrasses become anaemic is unknown. In rainbow trout *Oncorhynchus mykiss* (Walbaum) haematocrit normally ranges from 23 to 33% and values <22% are deemed anaemic (Gallaughier *et al.*, 1995). Considering median haematocrit was $32.37 \pm 4.90\%$ in control *H. melapterus* and the lowest haematocrit was 8.81% in infected fish, it is likely that some infected fish were anaemic and that continued exposure would result in mortality.

The number of isopods collected from infected fish was 278–1251 (mean \pm s.e. 497.17 ± 81.92) per fish. No isopods were recovered from control fish. Wild *H. melapterus* at Lizard Island are parasitized on average by 21.5 ± 4.7 gnathiids (Grutter & Poulin, 1998), hence, isopod density in the exposure treatment was much higher than natural levels. The number of isopods removed per exposed fish was significantly positively correlated with the numbers of days from when the experiment began ($r = 0.612$, $n = 12$, $P < 0.05$), indicating that depletion of the culture was not occurring, but instead, that the population of isopods may have increased during the study. Most isopods collected were first stage (mean \pm s.e. 330.58 ± 56.51) or second stage (mean \pm s.e. 162.58 ± 44.24) juveniles. Very few third stage juveniles were collected (mean \pm s.e. 4 ± 1).

No significant correlations existed between fish L_T and total isopods collected ($r = 0.148$, $n = 12$, $P > 0.05$), or between haematocrit of exposed fish and their isopod loads ($r = 0.148$, $n = 12$, $P > 0.05$) or when only isopods with a blood meal were considered ($r = 0.206$, $n = 12$, $P > 0.05$). The small sample size

($n=12$) and limited size range of the fish (13.60–23.90 cm L_T) was most likely responsible for the lack of a positive correlation normally observed between host length and parasite load (Grutter & Poulin, 1998). The lack of correlation between isopod load and haematocrit probably occurred because the isopods collected post-exposure represent only a fraction of the total number that fed on the fish during the experimental period. This is because most first and second stage juveniles from the *Gnathia* sp. culture at Lizard Island drop off *H. melapterus* within an hour of attachment to fish (Grutter, 2003).

This study was funded by the Australian Research Council (A.S.G.) We thank N. Pankhurst and C. Franklin for advice on experimental design, J. Pickering, R.L. Schlecht and two anonymous reviewers for their comments on the manuscript, and the staff of Lizard Island Research Station for their help with the experiment.

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