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Effect of *Argulus* infestation on oxygen consumption, total protein and glucose in three freshwater ornamental fishes

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Abstract
Fish lice of the genus *Argulus* are widespread crustacean ectoparasites and a recurrent health problem in the ornamental fish trade, yet their effects on host physiology and tissue biochemistry in commonly traded species are poorly documented. This study compared oxygen consumption, total tissue protein and tissue glucose between uninfected and *Argulus*-infected goldfish (*Carassius auratus*), sailfin molly (*Poecilia latipinna*) and common carp (*Cyprinus carpio*). Oxygen consumption was estimated from the decline in dissolved oxsygen in the holding water, protein was estimated by the Folin–Ciocalteu method and glucose by the anthrone method. Groups were compared with Welch's unequal-variance *t*-test, with Holm correction across species, and a two-way analysis of variance (species × infection status) was used to test the overall infection effect and its consistency among species. Infection was associated with a marked and consistent depletion of tissue biochemical reserves. Total protein and glucose were substantially lower in infected fish of all three species, the differences remained significant after multiplicity adjustment, and the standardised effects were very large. Infection status was the dominant source of variation in both variables, although the size of the decline differed among species. In contrast, oxygen consumption did not differ significantly between infected and uninfected fish; the direction of change varied among species and variability was high relative to the sample size. These results show that *Argulus* infection depletes protein and carbohydrate reserves in ornamental fishes, consistent with the metabolic cost of blood-feeding and stress, and they do not support the view that infection consistently reduces respiration. Larger, replicated studies with quantified parasite load, blood chemistry and histology are needed.

Keywords: Fish louse, Aquarium fishes, Host–parasite interaction, Tissue biochemistry, Respiration, Branchiura

1. Introduction

Ornamental fish keeping is one of the most popular aquarium hobbies, and the associated trade in live fish has become an important source of income and employment in many tropical countries, including India. Intensive holding, repeated handling and long-distance transport expose ornamental fishes to crowding and stress, conditions under which parasites that are otherwise tolerated can multiply and cause disease. Parasitic infections therefore remain one of the main constraints on the health, appearance and marketability of aquarium fishes.

Among the ectoparasites of freshwater fishes, the branchiuran crustaceans of the genus *Argulus*, commonly called fish lice, are of particular concern. They attach to the skin, fins and gill region of the host, pierce the epidermis and feed on mucus, epithelial cells and blood, causing local lesions, inflammation and a risk of secondary bacterial and fungal infection (Walker *et al*., 2004). Heavy infestations can produce mortality, and even light infestations are unwelcome in ornamental fishes because the lesions reduce their market value.

The physiological consequences of *Argulus* infestation have been examined mainly in food fishes. Haematological changes have been reported in parasitised pacu (Tavares-Dias *et al*., 1999), plasma constituents have been compared in infested common carp before and after treatment (Ranzani-Paiva *et al*., 1989), and serum glucose and immune parameters have been measured in rohu infested with *Argulus siamensis*, in which glucose was elevated while total protein did not differ between uninfested fish and those with moderate or heavy infestation (Saurabh and Sahoo, 2010). Ectoparasites can also raise the host's metabolic rate: naturally parasitised bridled monocle bream, for example, had a higher standard metabolic rate than unparasitised fish (Binning *et al*., 2013). Information on tissue-level biochemistry and respiration in commonly traded ornamental species is, by comparison, scarce, and the published findings are not uniform in direction.

The present study therefore compared oxygen consumption, total tissue protein and tissue glucose between normal (uninfected) and *Argulus*-infected individuals of three freshwater ornamental fishes: goldfish (*Carassius auratus*), sailfin molly (*Poecilia latipinna*) and common carp (*Cyprinus carpio*). The aims were to quantify the differences with appropriate statistical tests and effect sizes, and to examine whether the response of the three species to infection was consistent.

2. Materials and Methods

2.1 Fish and experimental groups

Three freshwater ornamental species were studied: goldfish *Carassius auratus*, sailfin molly *Poecilia latipinna* and common carp *Cyprinus carpio*. For each species, normal (uninfected) fish and fish infected with the fish louse *Argulus* sp. (family Argulidae) were compared. Three parameters were measured: oxygen consumption, total protein and glucose.

2.2 Oxygen consumption

Normal and infected fish were placed separately in a known volume of water. The dissolved oxygen of the water was determined before the fish were introduced and again after the exposure period, and the rate of oxygen consumption was calculated from the difference and expressed per unit body weight per hour (mg/body wt/h) for normal and infected fish.

2.3 Total protein

Tissue samples of 200 mg were taken from normal and infected fish, with five replicates per group. Total protein was estimated by the Folin–Ciocalteu method (Lowry *et al*., 1951).

2.4 Glucose

Body tissue samples of 200 mg were collected from normal and infected fish, and glucose was estimated colorimetrically by the anthrone method (Roe, 1955).

2.5 Statistical analysis

Results are presented as mean ± standard deviation (SD) with five replicates per group ($n = 5$). The analysis was carried out from these group summaries. For each species and parameter, normal and infected groups were compared with Welch's unequal-variance *t*-test (Welch, 1947), with Satterthwaite degrees of freedom, because the group variances differed markedly. The 95% confidence interval (CI) of the mean difference was based on the same *t* distribution. Because three species were tested for each parameter, *p*-values were adjusted by the Holm step-down procedure (Holm, 1979), and both unadjusted and adjusted values are reported. Standardised effect size was expressed as Hedges' *g* with an approximate 95% CI (Hedges, 1981), and the percentage change in infected fish relative to normal fish was also calculated.

To test the overall effect of infection and whether it was consistent across species, a balanced two-way analysis of variance (ANOVA) with species and infection status as fixed factors and their interaction was fitted for each parameter, using the pooled within-group variance from the group SDs (error df = 24). All tests were two-sided with significance accepted at $p < 0.05$.

3. Results

3.1 Oxygen consumption

Mean oxygen consumption of normal and infected fish is given in Table 1. Infected goldfish consumed less oxygen than normal goldfish (64.0% lower) and infected common carp also showed a lower mean (42.5% lower), whereas infected sailfin mollies showed a higher mean (152.3% higher). None of these differences was statistically significant (Table 2): goldfish, $t = 0.64$, $p = 0.550$; sailfin molly, $t = -1.28$, $p = 0.268$; common carp, $t = 0.85$, $p = 0.440$ (all Holm-adjusted $p \geq 0.80$). The confidence intervals of the mean differences included zero in every species, and the standard deviations were large relative to the means, particularly in goldfish and common carp (Figure 1). In the two-way ANOVA there was no significant effect of species ($F_{2,24} = 1.75$, $p = 0.195$), infection status ($F_{1,24} = 0.39$, $p = 0.538$) or their interaction ($F_{2,24} = 0.42$, $p = 0.663$; Table 3).

Table 1. Oxygen consumption, total protein and glucose (mean ± SD, $n = 5$ per group) in normal and *Argulus*-infected ornamental fishes, with percentage change in infected relative to normal fish

Species	Normal	Infected	Change (%)
Oxygen consumption (mg/body wt/h)			
Carassius auratus	0.0399 ± 0.0836	0.0144 ± 0.0312	−64.0
Poecilia latipinna	0.00055 ± 0.00011	0.00139 ± 0.00146	+152.3
Cyprinus carpio	0.00058 ± 0.00064	0.00033 ± 0.00007	−42.5
Total protein (per 200 mg tissue)			
Carassius auratus	21.88 ± 7.45	10.90 ± 0.96	−50.2
Poecilia latipinna	32.06 ± 6.85	9.88 ± 0.95	−69.2
Cyprinus carpio	24.16 ± 2.42	9.41 ± 1.46	−61.1
Glucose (per 200 mg tissue)			
Carassius auratus	28.93 ± 4.09	5.71 ± 3.13	−80.3
Poecilia latipinna	24.96 ± 6.34	6.62 ± 2.20	−73.5
Cyprinus carpio	33.55 ± 1.58	5.25 ± 3.30	−84.4

Change (%) = (infected − normal)/normal × 100. Negative values indicate lower values in infected fish.

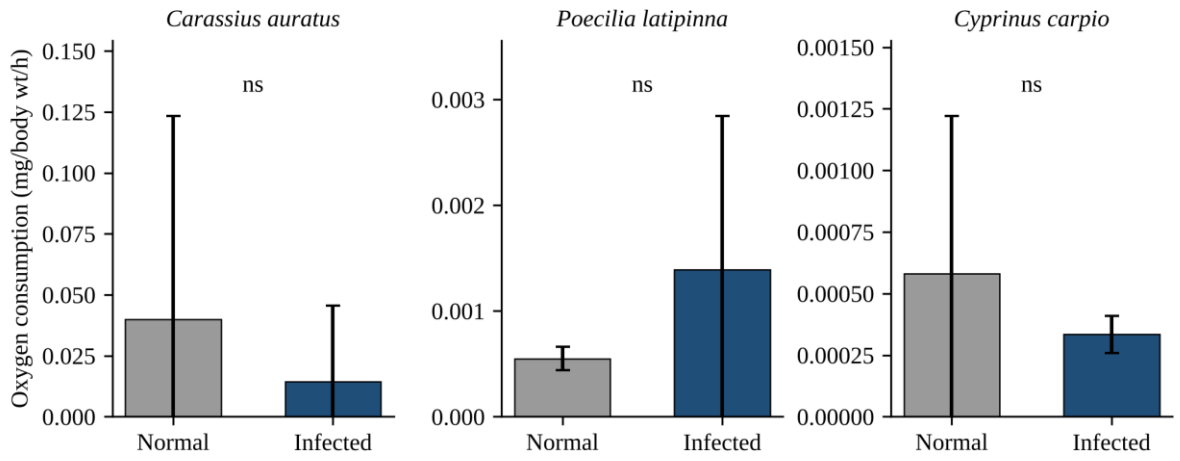


Figure 1. Oxygen consumption of normal and *Argulus*-infected goldfish, sailfin molly and common carp (mean ± SD, $n = 5$). Each panel has its own scale. ns, not significant (Holm-adjusted $p > 0.05$).

3.2 Total protein

Total protein was lower in infected fish than in normal fish in all three species (Table 1; Figure 2). The reduction was 50.2% in goldfish (21.88 ± 7.45 versus 10.90 ± 0.96; $t = 3.27$, df = 4.1, $p = 0.029$; Holm-adjusted $p = 0.029$), 69.2% in sailfin molly (32.06 ± 6.85 versus 9.88 ± 0.95; $t = 7.18$, df = 4.2, $p = 0.002$; adjusted $p = 0.003$) and 61.1% in common carp (24.16 ± 2.42 versus 9.41 ± 1.46; $t = 11.68$, df = 6.6, $p < 0.001$). All three differences were significant after Holm adjustment, and the standardised effects were very large (Hedges' g from 1.87 to 6.67; Table 2). Normal fish also showed greater between-replicate variation than infected fish, especially goldfish (SD 7.45 versus 0.96), which is why Welch's test was preferred to the pooled-variance test.

In the two-way ANOVA, infection status had a very strong effect on protein ($F_{1,24} = 102.27$, $p < 0.001$). There was also a significant effect of species ($F_{2,24} = 3.44$, $p = 0.048$) and a significant species × infection interaction ($F_{2,24} = 4.35$, $p = 0.024$; Table 3), indicating that the size of the decline was not identical in the three species: it was proportionally greatest in sailfin molly and smallest in goldfish, although the direction was the same in all.

Table 2. Welch's *t*-tests comparing normal and *Argulus*-infected fish for each parameter and species

Species	*t*	df	*p*	Holm *p*	Mean difference, 95% CI	Hedges' *g* (95% CI)
Oxygen consumption (mg/body wt/h)						
Carassius auratus	0.64	5.1	0.550	0.880	−0.07648 to 0.12749	0.37 (−0.89 to 1.62)
Poecilia latipinna	−1.28	4.0	0.268	0.804	−0.00264 to 0.00097	−0.73 (−2.02 to 0.56)
Cyprinus carpio	0.85	4.1	0.440	0.880	−0.00055 to 0.00104	0.49 (−0.77 to 1.75)
Total protein (per 200 mg tissue)						
Carassius auratus	3.27	4.1	0.029	0.029	1.76 to 20.19	1.87 (0.33 to 3.41)
Poecilia latipinna	7.18	4.2	0.002	0.003	13.73 to 30.65	4.10 (1.74 to 6.46)
Cyprinus carpio	11.68	6.6	< 0.001	< 0.001	11.73 to 17.78	6.67 (3.18 to 10.17)
Glucose (per 200 mg tissue)						
Carassius auratus	10.08	7.5	< 0.001	< 0.001	17.84 to 28.60	5.76 (2.68 to 8.84)
Poecilia latipinna	6.11	4.9	0.002	0.002	10.59 to 26.08	3.49 (1.38 to 5.60)
Cyprinus carpio	17.32	5.7	< 0.001	< 0.001	24.26 to 32.35	9.89 (4.89 to 14.90)

Mean difference = normal − infected (same units as Table 1), with 95% CI. Holm p = p-value adjusted across the three species within each parameter. Hedges' g is positive when the normal group is higher. Tests were computed from group means and SDs with $n = 5$ per group.

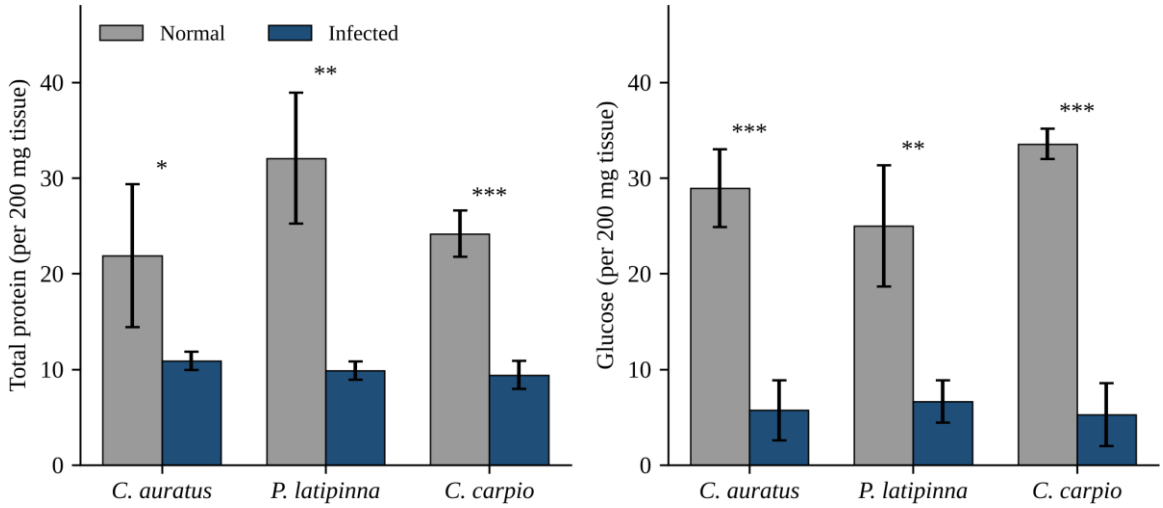


Figure 2. Total protein (left) and glucose (right) of normal and *Argulus*-infected fish (mean ± SD, $n = 5$). Asterisks give the Holm-adjusted significance of the normal–infected difference within each species: p < *0.05,* p < *0.01,* * p* < 0.001.

3.3 Glucose

Glucose showed the largest and most uniform change (Table 1; Figure 2). Infected fish had 80.3% less glucose than normal fish in goldfish (28.93 ± 4.09 versus 5.71 ± 3.13; $t = 10.08$, df = 7.5, $p < 0.001$), 73.5% less in sailfin molly (24.96 ± 6.34 versus 6.62 ± 2.20; $t = 6.11$, df = 4.9, $p = 0.002$) and 84.4% less in common carp (33.55 ± 1.58 versus 5.25 ± 3.30; $t = 17.32$, df = 5.7, $p < 0.001$). All three remained significant after Holm adjustment (adjusted $p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively), with very large effect sizes (Hedges' g 3.49 to 9.89; Table 2).

The two-way ANOVA showed a very strong main effect of infection status on glucose ($F_{1,24} = 287.18$, $p < 0.001$), no significant main effect of species ($F_{2,24} = 2.31$, $p = 0.121$) and a significant interaction ($F_{2,24} = 4.39$, $p = 0.024$; Table 3). The interaction reflects differences in the baseline and in the magnitude of the fall among species rather than any reversal: glucose was lower in infected fish in every species.

Table 3. Two-way ANOVA (species × infection status) for each parameter

Source of variation	df	*F*	*p*
Oxygen consumption (mg/body wt/h)			
Species	2, 24	1.75	0.195
Infection status	1, 24	0.39	0.538
Species × infection	2, 24	0.42	0.663
Total protein (per 200 mg tissue)			
Species	2, 24	3.44	0.048
Infection status	1, 24	102.27	< 0.001
Species × infection	2, 24	4.35	0.024
Glucose (per 200 mg tissue)			
Species	2, 24	2.31	0.121
Infection status	1, 24	287.18	< 0.001
Species × infection	2, 24	4.39	0.024

Error df = 24 for all tests (five replicates in each of six species × infection cells).

4. Discussion

4.1 Overview

The clearest result of this study is that *Argulus* infection was accompanied by lower tissue protein and glucose in all three ornamental species, with effects large enough to remain significant after correction for multiple testing and with consistent direction across species. By contrast, no reliable effect on oxygen consumption could be demonstrated. The two sets of findings have different implications and are discussed separately.

4.2 Protein

Lower tissue protein in infected fish is consistent with the biology of the parasite. *Argulus* feeds on blood, mucus and epidermal cells and damages the skin at the attachment and feeding sites (Walker *et al*., 2004), so the host

loses protein directly through feeding and through the repair of lesions and mucus replacement. The host's response to the stress of infestation may in addition shift protein towards energy production. Whichever pathway predominates, the data indicate that infected fish carry a smaller protein reserve than uninfected fish. This differs from the report that total serum protein of rohu did not change under moderate and heavy infestation (Saurabh and Sahoo, 2010), but the two findings are not directly comparable because we measured whole-tissue protein, whereas their measurement was of serum, a compartment that is regulated more tightly and responds to immune activation as well as to nutritional state.

4.3 Glucose

The fall in tissue glucose was the largest and most uniform change observed. A plausible interpretation is that infected fish draw on their carbohydrate reserves to meet the energetic cost of stress, tissue repair and immune responses, and that feeding and activity are reduced during infestation. Elevated serum glucose has been reported in fish infested with *Argulus* (Saurabh and Sahoo, 2010), which is the opposite direction to our finding. Circulating glucose rises acutely under stress as a result of mobilisation of reserves, whereas the tissue glucose measured here may reflect the depletion that follows; our measurements cannot separate the two, and a comparison with plasma glucose, glycogen and cortisol measured in the same individuals would be needed to resolve the question. Plasma glucose and total protein of common carp have also been compared before and after treatment of *Argulus* infestation without significant change (Ranzani-Paiva *et al*., 1989), again underlining that findings differ with the tissue examined, the parasite load and the time course of infection.

4.4 Oxygen consumption

Infected goldfish and common carp had lower mean oxygen consumption and infected sailfin mollies higher mean consumption, but none of the differences was significant and the intervals were wide. The direction was therefore not consistent, and the data do not support a general conclusion that *Argulus* infection reduces respiration. Higher oxygen consumption in parasitised fish has been reported in other host–ectoparasite systems and attributed to the additional cost of maintenance and parasite drag (Binning *et al*., 2013), whereas damage to the gills could be expected to limit oxygen uptake; both effects could operate, depending on the species, the site and intensity of attachment and the time since infection, and could offset each other in a small sample. The large standard deviations, especially in goldfish and common carp, suggest that variation among individual fish and in the experimental conditions swamped any effect of infection. Because the test has little power with five replicates, the non-significant result should be read as inconclusive rather than as evidence of no effect.

4.5 Species differences and limitations

The significant species × infection interaction for protein and glucose shows that the three species do not respond identically, although the direction of change was the same in all. Interpretation of these differences is limited because baselines also differed among species. The study has several other limitations. The replication is small (n = 5 per group), and the analysis was based on group means and SDs rather than on individual values, which prevented checks of normality and outlying observations; the Welch test is robust to unequal variance but not to marked departures from normality in very small samples. Parasite load (number of *Argulus* per fish) was not used as a covariate, so dose–response relationships could not be examined. Protein and glucose were measured in whole-tissue samples at a single time, and the results cannot indicate whether the changes are a cause or a consequence of infection or how quickly they develop. No blood chemistry, haematology or histology was included. Finally, oxygen consumption showed very high between-replicate variability, which, together with the small sample, limits what can be concluded about respiration.

4.6 Implications

For the ornamental fish trade, depletion of protein and glucose reserves in infected fish is a physiological cost that may reduce growth, resistance to other stressors and survival during holding and transport. This supports routine inspection and quarantine of newly acquired fish and early treatment of infestations, which are inexpensive compared with the losses that follow an outbreak. Future work should combine experimental infection at known parasite loads with repeated sampling, blood and tissue biochemistry, and respirometry under controlled conditions and with larger replication.

5. Conclusions

Argulus infection was associated with significantly lower tissue protein and glucose in goldfish, sailfin molly and common carp, and infection status explained most of the variation in these two variables, although the size of the effect differed among species. Oxygen consumption did not differ significantly between infected and normal fish, and its direction of change was not consistent across species. The results indicate that infestation depletes the biochemical reserves of ornamental fishes, and they underline the need for larger, better-replicated studies that record parasite load and include blood and histological measures.

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Declarations

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