Journal of FISH BIOLOGY

*Journal of Fish Biology* (2015) **87**, 836–847
doi:10.1111/jfb.12749, available online at wileyonlinelibrary.com

# Measuring fish body condition with or without parasites: does it matter?

C. Lagrue* and R. Poulin

*University of Otago, Department of Zoology, Dunedin, 340 Great King Street, P.O. Box 56, Dunedin 9054, New Zealand*

*(Received 23 March 2015, Accepted 11 June 2015)*

A fish body condition index was calculated twice for each individual fish, including or excluding parasite mass from fish body mass, and index values were compared to test the effects of parasite mass on measurement of body condition. Potential correlations between parasite load and the two alternative fish condition index values were tested to assess how parasite mass may influence the perception of the actual effects of parasitism on fish body condition. Helminth parasite mass was estimated in common bully *Gobiomorphus cotidianus* from four New Zealand lakes and used to assess the biasing effects of parasite mass on body condition indices. Results showed that the inclusion or exclusion of parasite mass from fish body mass in index calculations significantly influenced correlation patterns between parasite load and fish body condition indices. When parasite mass was included, there was a positive correlation between parasite load and fish body condition, seemingly indicating that fish in better condition supported higher parasite loads. When parasite mass was excluded, there was no correlation between parasite load and fish body condition, *i.e.* there was no detectable effect of helminth parasites on fish condition or fish condition on parasite load. Fish body condition tended to be overestimated when parasite mass was not accounted for; results showed a positive correlation between relative parasite mass and the degree to which individual fish condition was overestimated. Regardless of the actual effects of helminth parasites on fish condition, parasite mass contained within a fish should be taken into account when estimating fish condition. Parasite tissues are not host tissues and should not be included in fish mass when calculating a body condition index, especially when looking at potential effects of helminth infections on fish condition.

Key words: body condition index; *Gobiomorphus cotidianus*; helminth parasites; parasite load index; residual index.

## INTRODUCTION

Fishes are hosts to taxonomically diverse helminth parasites and infections can significantly affect fish behaviour, metabolism, body condition, fecundity and survival (Barber *et al.*, 2000; Lafferty, 2008; Seppänen *et al.*, 2009). A simple and common method by which the pathological effects of parasites on fishes can be estimated involves the use of a variety of body condition indices ($I_{BC}$; Bolger & Connolly, 1989). The study of condition generally uses length and mass relationships, assuming that heavier fish of a given length are in better condition (Jones *et al.*, 1999). Body

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*Author to whom correspondence should be addressed. Tel.: +64 3479 5848; email: clement.lagrue@gmail.com

condition indices based on length and mass relationships are easy and cheap to obtain, requiring only the fish body length and total mass to calculate. These measurements are non-intrusive and can be obtained rapidly in the field and on live fish without the need for elaborate equipment or fish dissection. Alternative indices, independent of fish size and mass, exist to assess fish condition. These include, but are not limited to, measures of fat or protein content, lipid composition or RNA:DNA ratios (Hails, 1983; Lemly & Esch, 1984; Fraser, 1989; Buckley *et al.*, 1999; Vasconcelos *et al.*, 2009). These indices often require time-consuming and expensive laboratory analyses (Bolger & Connolly, 1989). Consequently, $I_{BC}$ based on length and mass relationships remain largely used.

Parasites are often expected to reduce fish body condition, infected individuals being leaner than uninfected ones due to pathological effects of parasites. Heavily infected fishes should thus have a lower $I_{BC}$ than their uninfected conspecifics, or individuals infected with fewer parasites, if pathological effects increase with infection intensity (Lemly & Esch, 1984; Santoro *et al.*, 2013). Changes in fish body condition, based on length and mass indices, can simply reflect normal seasonal fluctuations, sex differences, spawning cycles and even fullness of the gut (Weatherly, 1972). Basically, fishes with a full stomach will be given higher $I_{BC}$ than conspecifics with identical body length and somatic mass but empty stomachs, even if they are actually in similar condition. Differences in satiety should thus be accounted for when estimating and comparing $I_{BC}$.

Similarly, parasite mass (total mass of all parasites found within an individual fish) is almost always ignored in fish $I_{BC}$ calculation. At the individual fish level, parasite mass is often only considered in systems where relatively large parasites infect small fish. For example, a single individual of the cestode *Schistocephalus solidus* can be proportionally large compared with its host, the three-spined stickleback *Gasterosteus aculeatus* L. 1758 (Tierney *et al.*, 1996). In contrast, the vast majority of parasites have proportionally very small body sizes compared with the fish they infect. Fishes, however, can be infected by many parasites that may be individually small, and thus seem insignificant size-wise compared with their fish host, but add up to a non-negligible total mass (Santoro *et al.*, 2013). Heavier fishes may seem in better condition but, alternatively, could be more heavily infected (*i.e.* harbour higher parasite loads). Fish individuals of equivalent body length can also be of similar total mass (fish mass + parasite mass), thus yielding equivalent $I_{BC}$ values, but may actually harbour greatly different parasite loads. Although parasites are contained within the host body, ultimately, parasite tissue is not fish tissue and should be excluded from fish mass when estimating fish $I_{BC}$.

While other pathological effects of some parasites on fishes can be genuinely severe, estimating their impact on fish using $I_{BC}$ may be prone to error if parasite mass itself is not taken into account during index estimation. Here, data collected were used to test for potential bias induced by the total mass of parasites (parasite load) harboured by a fish when calculating $I_{BC}$ and whether this may influence our assessment of the effects of parasites on fish condition.

## MATERIALS AND METHODS

### FIELD SAMPLING AND LABORATORY PROCESSING

The common bully *Gobiomorphus cotidianus* McDowall 1975 was used to test for potential effects of parasites on fish body condition. *Gobiomorphus cotidianus* is a small, benthic fish, widely distributed in New Zealand and very abundant in littoral habitats of lakes and slow flowing rivers. It commonly reaches 110 mm in fork length ($L_F$; 150 mm maximum) although lake fish tend to be smaller (70–80 mm adult size; McDowall, 1990). It is relatively short-lived (*c*. 3 years) and after a pelagic larval phase, fish of all size and ages are found sharing the same habitats (McDowall, 1990). This species consumes a wide diversity of prey and forms an important food resource for other fish and bird species. As a result, *G. cotidianus* is host to a variety of both juvenile (trematode metacercariae and nematode larvae) and adult helminth parasites (Lagrue *et al.*, 2011; Herrmann & Poulin, 2011). Gut adult parasites are acquired by feeding on infected prey (trophic transmission) that serve as intermediate hosts to some of these parasites (Marcogliese, 2002; Parker *et al.*, 2003); *G. cotidianus* is thus used as a definitive host by these species. Helminth larval stages can be acquired similarly through consumption of infected prey, although, in the systems studied, most parasites are trematodes that infect fish directly by skin penetration. Larval helminths use *G. cotidianus* as an intermediate host for transmission to their definitive host, fish-eating birds or larger fishes, depending on the parasite species.

Fish were sampled in four lake ecosystems. Lake Hayes (44°58′59.4″S; 168°48′19.8″E), Lake Tuakitoto (46°13′42.5″S; 169°49′29.2″E), Lake Waihola (46°01′14.1″S; 170°05′05.8″E) and Tomahawk Lagoon (45°54′06.0″S; 170°33′02.2″E), South Island, New Zealand, were selected to provide a variety of lake types (size, depth and altitude), freshwater communities (coastal *v*. alpine, oligotrophic *v*. eutrophic and tidal or not) and local avian communities. The four lakes were sampled in early spring, summer and late autumn (austral seasons: September 2012, January and May 2013). In each lake and during each season (4 lakes × 3 seasons = 12 samples), *G. cotidianus* were sampled to assess potential temporal and spatial variability among lakes in fish body condition and parasite burden.

Combinations of fish catching gear types were used so that accurate cross sections of *G. cotidianus* size classes were sampled from each lake in each season. First, eight fyke nets and 40 minnow traps were set in the evening along the littoral zone of the lakes. Fyke nets and minnow traps were set during the night, when fish are more active, as they are passive sampling methods relying on fish to willingly encounter and enter traps (Hubert, 1996). The next day, trapped fish were recovered and killed for later dissection. Fish sampling was then complemented using a standard, fine-mesh purse seine (5 mm mesh size). As an active sampling method, seining captures small and less mobile individuals that are not captured by passive gear like fyke nets and minnow traps (Nielsen & Johnson, 1983). All fish were killed immediately to inhibit the digestion process, and stored on ice to preserve internal tissues, stomach contents and parasites for future identification, count and measures. Fish were killed by severing the spinal cord with scissors and destroying the brain stem, following University of Otago Animal Ethics Committee guidelines. Death was instantaneous and confirmed by the absence of eye reflex and gill movements.

In the laboratory, fish were measured to the nearest mm ($L_F$), weighed to the nearest 0.01 g (total fish mass) and then dissected. Their gut, from oesophagus to anus, and all internal organs and tissues were removed and preserved in 70% ethanol for later diet and parasite analyses. Eviscerated fish bodies were frozen individually until dissection.

Complete necropsies of all fish were conducted under a dissecting microscope. The head, gills, eyes, brain and spine of each fish were examined using fine forceps to pull apart fish tissues and obtain a precise estimation of parasite load for each fish. Soft tissues (muscle and skin) were removed from the spine, crushed between two glass plates and examined by transparency to identify and count parasites. Internal organs and tissues and the gut were first rinsed in water to wash off the ethanol. The gut was then separated from other organs and tissues. Liver, swimbladder, gall bladder, gonads and other organs and tissues from the body cavity (fat, mesentery, kidneys and heart) were all screened for parasites. Finally, the gut was dissected, contents were removed, screened for parasites and then set aside for diet examination. Oesophagus, stomach, intestine and rectum were then examined for gut parasites. All parasites were identified, counted and a subsample of 20 individuals per species (or all individuals when < 20 were found in a fish) were measured to the nearest 0.01 mm (diameter for spherical parasites, such as encysted trematode metacercariae; length, width and thickness for flattened ellipsoids, like adult trematodes; length and width for cylindrical parasites such as nematodes).

## PARASITE AND PREY MASS

Body mass was estimated differently for prey or parasite taxa. Parasites were too small to be individually weighed and body measurements indicated that they varied little in size within species. Body volume was thus calculated for the subsamples of parasites measured during fish dissection based on the most appropriate formula for the shape of each species (*e.g.* nematodes = cylinder, adult and non-encysted juvenile trematodes = flattened ellipsoid, encysted juvenile trematodes = spheres). A mean body volume was then calculated for each species and this volume ($mm^3$) was converted to mass (mg) assuming the parasite density equalled that of water (Santoro *et al.*, 2013).

Most prey taxa found in the fish diet were large enough to be weighed individually (fishes, terrestrial insects, isopods, chironomids, odonates, trichopteran larvae, adult hemipterans, molluscs and leeches). Prey items varied little in size within taxon and by weighing a subsample of individuals for each taxon (to the nearest 0.01 mg) the mean body mass of an individual could be calculated for each prey taxa. For small invertebrate prey, which varied little in size intraspecifically (amphipods, oligochaetes, planktonic crustaceans and small insect larvae), five, 10 or 20 conspecific individuals were pooled (depending on individual body size), weighed them as a group and, from the total mass recorded, calculated the average body mass of one individual.

For each prey and parasite taxon, mass was simply the product of the mean individual body mass of each taxon by the number of individuals of that particular taxon found in each individual fish. Total diet content and parasites masses were then calculated by adding the mass of all prey or parasite taxa found within each fish. Total diet content mass and total parasite mass were thus obtained for each individual fish. Total parasite mass was used because mass is expected to reflect potential costs of parasite infections to the fish more accurately, and accounts for size differences among helminth species (Muzzall & Peebles, 1998, Neff & Cargnelli, 2004).

## FISH BODY CONDITION AND PARASITE LOAD INDICES

The residual index was used as fish $I_{BC}$ (Jakob *et al.*, 1996). Fish body mass was regressed on fish body length (recorded $L_F$) after the data were ln transformed to meet the assumptions of regression. The residual distances of individual points from this regression line then served as the estimators of body condition, i.*e*. whether an individual fish's body condition was above or below average for its length (Cone, 1989; Neff & Cargnelli, 2004). Fish body condition can be influenced by the state of fullness of the gut (Bolger & Connolly, 1989). To eliminate potential effects of fish satiety on $I_{BC}$, fish body mass used to estimate $I_{BC\ total}$ was calculated as: total fish mass (*i.e.* recorded mass) minus diet content mass. Furthermore, to test for potential confounding effects of parasite mass on fish body condition estimation, an alternative fish $I_{BC}$ ($I_{BC\ parasite}$) was concomitantly calculated using the parasite-free fish body mass calculated as: total fish mass minus (diet content mass plus parasite mass). The difference between fish body condition indices calculated with ($I_{BC\ total}$) and without ($I_{BC\ parasite}$) parasite mass was also estimated for each individual ($\Delta I_{BC} = I_{BC\ total} - I_{BC\ parasite}$). Proportionally, the higher the parasite load within a host, the more its actual body condition is likely to be overestimated if parasite mass is not taken into account.

A parasite load index ($I_{PL}$) was estimated in the same way as fish $I_{BC}$. Parasite mass was regressed on $L_F$ after ln transformations. The residual distances of individual points from this regression line then served as estimators of parasite load in individual hosts (*i.e.* $I_{PL}$), that is, whether an individual host harbours more or less parasite mass than average for its body length. Here, only one index can be calculated for each fish since there is only one estimate of parasite mass per individual. In addition, the proportion of fish body mass actually constituted of parasite tissue within each individual fish was estimated. The proportion of parasite in fish body mass was calculated as: parasite mass divided by (total fish mass minus diet content mass).

## DATA ANALYSIS

$L_F$ varied significantly among sampled lakes (mean ± S.D. = 49.1 ± 15.4, 67.5 ± 26.4, 39.9 ± 9.7 and 47.6 ± 7.9 mm in Hayes, Tomahawk, Waihola and Tuakitoto; ANOVA, $F_{3,257} = 24.279$, $P < 0.001$). Potential effects of sampling site (lake) and season on fish and parasite mass were thus controlled for $L_F$ using ANCOVAs with $L_F$ included as a covariate.

Sampling season had no effect on either total fish mass (*i.e.* recorded mass) or parasite mass (ANCOVA, $F_{2,248} = 1.395$ and 2.908, both $P > 0.05$). Fish mean ± S.D. mass (2.17 ± 2.34, 6.54 ± 6.06, 0.84 ± 0.66 and 1.43 ± 0.96 g in Hayes, Tomahawk, Waihola and Tuakitoto) and mean parasite mass per fish (Table I) varied significantly among lakes (ANCOVA, $F_{3,247} = 13.328$ and 7.936, both $P < 0.001$). $I_{BC}$ and $I_{PL}$ were therefore calculated separately for each lake but seasonal data were combined within lakes to increase sample size. The regressions of ln-transformed fish body mass, parasite-free fish body mass and parasite mass on ln-transformed $L_F$ were highly significant in all four lakes (Table II). Both fish body masses and parasite mass were positively correlated with $L_F$ (Figs 1 and 2). The residuals from these analyses were extracted to be used as $I_{BC}$ ($I_{BC\ total}$ and $I_{BC\ parasite}$) and $I_{PL}$ for each individual fish.

Potential size bias was tested for each index by checking whether the $I_{BC}$ and $I_{PL}$ correlated with $L_F$, using Pearson's *r*. These indices should show no correlation with $L_F$ (Jakob et al. 1996). $I_{BC}$ and $I_{PL}$ of all individual fish from all four lakes were combined to test for general potential correlations between $I_{PL}$ and $I_{BC\ total}$ and $I_{BC\ parasite}$, using Pearson's *r*. Finally, the potential correlation between $\Delta I_{BC}$ correlated and the proportion of parasite mass in fish body mass was tested.

## RESULTS

Seven different species of helminth parasites were found infecting *G. cotidianus* (Table I). Four juvenile trematodes (metacercariae) and one juvenile nematode (larva) used *G. cotidianus* as an intermediate host and rely on trophic transmission to reach their definitive host. *Stegodexamene anguillae* and *Telogaster opisthorchis* are gut parasites of eels (*Anguilla* sp.) while the three others (*Apatemon* sp., *Tylodelphis* sp. and *Eustrongylides* sp.) infect the digestive system of freshwater birds (Table I). Adult *Deretrema* sp. and *Coitocaecum parvum* use *G. cotidianus* as their definitive host; fish acquire these parasites when ingesting infected crustacean intermediate hosts.

There was no size bias for any of the three indices; $I_{BC\ total}$, $I_{BC\ parasite}$ and $I_{PL}$ did not correlate with $L_F$ ($r^2 = 0.0012$, 0.0013 and 0.0010, d.f. = 259, all $P > 0.05$). Overall, there was a weak but significantly positive correlation between $I_{PL}$ and $I_{BC\ total}$ ($r^2 = 0.0374$, d.f. = 259, $P < 0.01$). Fish in better body condition (higher $I_{BC\ total}$) seemed to carry more parasites [higher $I_{PL}$; Fig. 3(a)], independently of their body length. When parasite mass was taken into account, there was no correlation between fish body condition ($I_{BC\ parasite}$) and $I_{PL}$ [$r^2 = 0.0003$, d.f. = 259, $P > 0.05$; Fig. 3(b)].

There was an overall positive relationship between $\Delta I_{BC}$ and the proportion of host mass actually made of parasite tissue [$r^2 = 0.7062$, d.f. = 259, $P < 0.001$; Fig. 4(a)]. Proportionally, the greater the parasite mass contained within a fish, the more its body condition was overestimated if parasite mass was not taken into account. There seemed, however, to be a threshold below which parasite mass did not affect $\Delta I_{BC}$. When parasites make up < 3% of the total fish mass, the proportion of parasite tissue in fish mass did not affect $I_{BC}$ [$r^2 = 0.0004$, d.f. = 196, $P > 0.05$; Fig. 4(b)]. Above 3%, there was a clear correlation between the proportion of the host mass represented by parasites and the overestimation of host body condition; $\Delta I_{BC}$ increased linearly with the proportion of parasite tissue in fish mass [$r^2 = 0.9467$, d.f. = 61, $P < 0.001$; Fig. 4(c)].

## DISCUSSION

The data showed that parasite mass can have a confounding effect in fish $I_{BC}$ estimation. In the present study, when parasite mass was not accounted for (*i.e.* included

TABLE I. Parasite class, life stage [juvenile (metacercaria or larva) or adult; encysted or active], transmission mode to the fish, main infection sites, mean individual mass and mean ± S.D. parasite mass per individual fish of each parasite species found in *Gobiomorphus cotidianus* in the four lakes sampled. Mean ± S.D. total parasite mass (mass of all parasites combined) per fish is also indicated

| Species | *Apatemon* sp. | *Stegodexamene anguillae* | *Telogaster opisthorchis* | *Tylodelphys* sp. | *Eustrongylides* sp. | *Deretrema* sp. | *Coitocaecum parvum* | Total |
|---|---|---|---|---|---|---|---|---|
| Class | Trematoda | Trematoda | Trematoda | Trematoda | Nematoda | Trematoda | Trematoda | |
| Life stage | Metacercaria (encysted) | Metacercaria (encysted) | Metacercaria (encysted) | Metacercaria (active) | Larva (encysted) | Adult (active) | Adult (active) | |
| Transmission | Direct | Direct | Direct | Direct | Trophic | Trophic | Trophic | |
| Infection sites | Muscles, body cavity, connective tissues | Muscles, gonads, connective tissues, body cavity | Muscles, gonads, connective tissues, body cavity | Eyes | Body cavity | Gall bladder | Intestine | |
| Mean individual mass (mg) | 0.103 | 0.478 | 0.091 | 0.032 | 4.169 | 0.189 | 0.052 | |
| Lake | | | | Parasite mass (mg) per individual fish | | | | |
| Hayes | 41.71 ± 50.57 | 5.833 ± 6.976 | 0.670 ± 1.321 | 0.318 ± 0.398 | 2.293 ± 4.504 | 0 ± 0 | 0 ± 0 | 50.82 ± 57.19 |
| Tomahawk | 232.1 ± 416.2 | 39.260 ± 54.350 | 3.126 ± 4.996 | 0 ± 0 | 6.160 ± 9.975 | 0 ± 0 | 0.180 ± 0.321 | 280.80 ± 520.70 |
| Waihola | 7.95 ± 9.39 | 4.098 ± 5.372 | 0.747 ± 1.130 | 0 ± 0 | 0.560 ± 1.762 | 0.039 ± 0.096 | 0.310 ± 0.428 | 13.70 ± 17.51 |
| Tuakitoto | 11.77 ± 13.69 | 0.843 ± 0.973 | 0.344 ± 0.712 | 0 ± 0 | 0.181 ± 0.869 | 0 ± 0 | 0.090 ± 0.082 | 13.23 ± 14.61 |

TABLE II. Linear regression analysis of (a) ln fish body mass ($M_{\mathrm{F}}$) and ln parasite-free fish body mass ($M_{\mathrm{F}-M_{\mathrm{P}}}$) and (b) ln parasite mass ($M_{\mathrm{P}}$), against ln fork length ($L_{\mathrm{F}}$) for *Gobiomorphus cotidianus* in the four lakes sampled

| (a) Lake | Regression equation | ANOVA summary | $r^2$ |
|---|---|---|---|
| Hayes | $M_{\mathrm{F}} = 3.28L_{\mathrm{F}} - 4.79$ | $F_{1,58} = 8859$, $P < 0.001$ | 0.9935 |
| | $M_{\mathrm{F}-M_{\mathrm{P}}} = 3.27L_{\mathrm{F}} - 4.81$ | $F_{1,58} = 9676$, $P < 0.001$ | 0.9940 |
| Tomahawk | $M_{\mathrm{F}} = 3.26L_{\mathrm{F}} - 4.81$ | $F_{1,109} = 20\,664$, $P < 0.001$ | 0.9948 |
| | $M_{\mathrm{F}-M_{\mathrm{P}}} = 3.23L_{\mathrm{F}} - 4.79$ | $F_{1,109} = 15\,806$, $P < 0.001$ | 0.9932 |
| Waihola | $M_{\mathrm{F}} = 3.27L_{\mathrm{F}} - 4.93$ | $F_{1,65} = 5120$, $P < 0.001$ | 0.9875 |
| | $M_{\mathrm{F}-M_{\mathrm{P}}} = 3.27L_{\mathrm{F}} - 4.94$ | $F_{1,65} = 4750$, $P < 0.001$ | 0.9865 |
| Tuakitoto | $M_{\mathrm{F}} = 3.31L_{\mathrm{F}} - 4.92$ | $F_{1,21} = 615.9$, $P < 0.001$ | 0.9670 |
| | $M_{\mathrm{F}-M_{\mathrm{P}}} = 3.30L_{\mathrm{F}} - 4.92$ | $F_{1,21} = 618.6$, $P < 0.001$ | 0.9672 |

| (b) Lake | Regression equation | ANOVA summary | $r^2$ |
|---|---|---|---|
| Hayes | $M_{\mathrm{P}} = 4.03L_{\mathrm{F}} - 9.93$ | $F_{1,58} = 137.6$, $P < 0.001$ | 0.7034 |
| Tomahawk | $M_{\mathrm{P}} = 4.29L_{\mathrm{F}} - 10.52$ | $F_{1,109} = 615.1$, $P < 0.001$ | 0.8495 |
| Waihola | $M_{\mathrm{P}} = 3.77L_{\mathrm{F}} - 9.99$ | $F_{1,65} = 146.2$, $P < 0.001$ | 0.6922 |
| Tuakitoto | $M_{\mathrm{P}} = 3.74L_{\mathrm{F}} - 10.34$ | $F_{1,21} = 56.83$, $P < 0.001$ | 0.7302 |

within fish mass), $I_{\mathrm{BC}}$ and $I_{\mathrm{PL}}$ were positively correlated. Fish in better body condition (*i.e.* heavier for a given length) seemed to harbour higher parasite loads [Fig. 3(a)]. Such a pattern could be interpreted as parasite-induced mortality in fish in poor body condition, with only individuals in good condition able to support high parasite burdens. When parasite mass was accounted for, this pattern disappeared and no relationship $I_{\mathrm{BC}}$

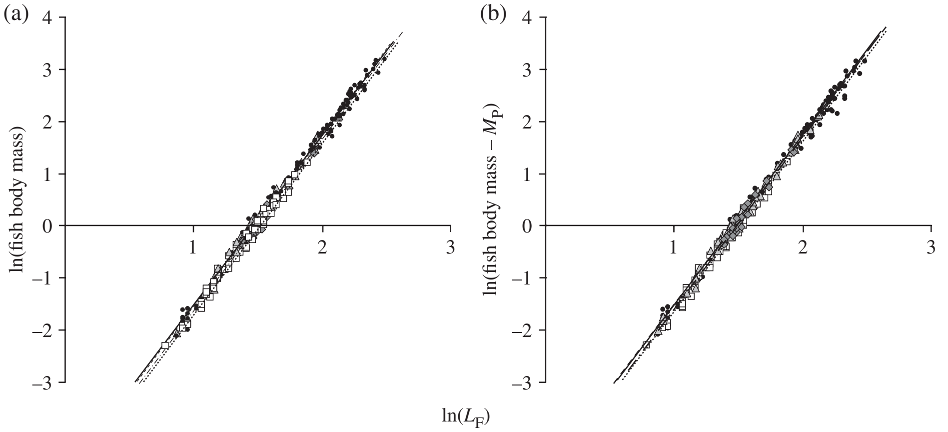

FIG. 1. Regression of (a) fish body mass ($M_{\mathrm{F}}$) and (b) parasite-free fish body mass ($M_{\mathrm{F}}$ - $M_{\mathrm{P}}$) on fish fork length ($L_{\mathrm{F}}$) of *Gobiomorphus cotidianus* in the four lakes sampled during our study (△, Hayes; ●, Tomahawk; □, Waihola; ◆, Tuakitoto). Regression lines are shown for each lake on each figure (____, Hayes; ........, Tomahawk; ............, Waihola; _._._., Tuakitoto); see Table II for regression equations and coefficients of determination. The residuals of points from the regression lines form the residual indices referred to as fish body condition indices, $I_{\mathrm{BCtotal}}$ from (a) and $I_{\mathrm{BCparasite}}$ from (b), respectively. $M_{\mathrm{P}}$, parasite mass.

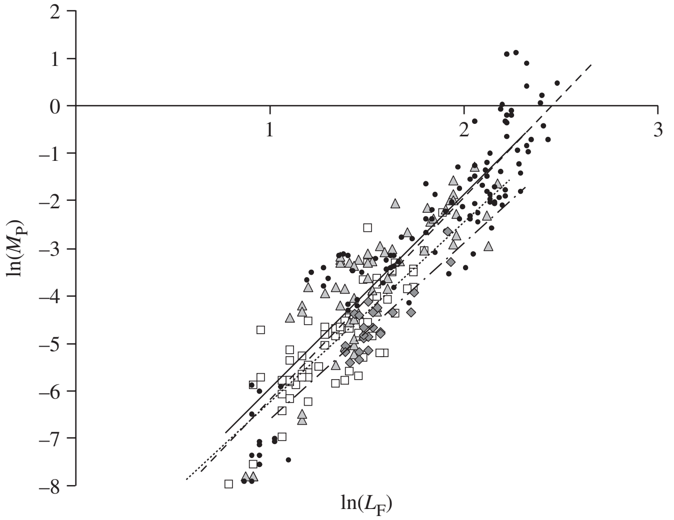

FIG. 2. Regression of parasite mass on *Gobiomorphus cotidianus* fork length in the four lakes sampled during our study (△, Hayes; ●, Tomahawk; □, Waihola; ◆, Tuakitoto). Regression lines are shown for each lake (——, Hayes; ........, Tomahawk; ..........., Waihola; -.-.-., Tuakitoto); see Table II for regression equations and coefficients of determination. The residuals of points from the regression lines form the residual index referred to as parasite load index ($I_{PL}$) in the text.

and $I_{PL}$ was found [Fig. 3(b)]. Body condition was not affected by helminth parasite load, and fish in better condition did not harbour higher parasite burdens in *G. cotidianus*. The results also show a clear, positive correlation between relative parasite mass and the degree to which individual fish $I_{BC}$ was overestimated (Fig. 4). The removal of parasite mass from fish mass did not affect $I_{BC}$ estimations in individuals with low parasite loads [parasite mass < 3% of fish mass; Fig. 4(b)]. Above that threshold, $I_{BC}$ was overestimated in proportion to the percentage of fish mass actually made up by parasite tissue [Fig. 4(c)]. Although the 3% threshold was arbitrarily chosen and may vary with fish and parasite taxa, and individual mass, parasite mass effects on fish $I_{BC}$ estimations are likely to be noticeable only beyond a certain threshold of parasite mass relative to fish mass.

Level of infection, parasite size, life stage, parasite taxa and the tissue or organ affected (site of infection) can all influence the pathological effects of parasites on the fish host (Pennycuick, 1971; Williams & Jones, 1994; Secombes & Chappell, 1996; Santoro *et al.*, 2013). Trematode parasites infecting *G. cotidianus* are individually small compared to the fish (Table I), and did not seem to induce lesions or inflammatory reactions in host tissues; no obvious tissue damage or inflammation was observed during dissections. Adult trematodes using *G. cotidianus* as definitive host actively feed on the fish host but were found in low numbers and are unlikely to have any significant pathological effect on the fish. Juvenile stages (*i.e.* metacercariae) of *Tylodelphys* sp. infect the eyes of fish where they remain active; they do not form a cyst like numerous trematode metacercariae. Trematodes with similar eye-infecting metacercariae (*Diplostomum* sp.) can have drastic effects on their fish host. *Diplostomum* sp. infection can impair vision and reduce feeding efficiency in fishes, consequently

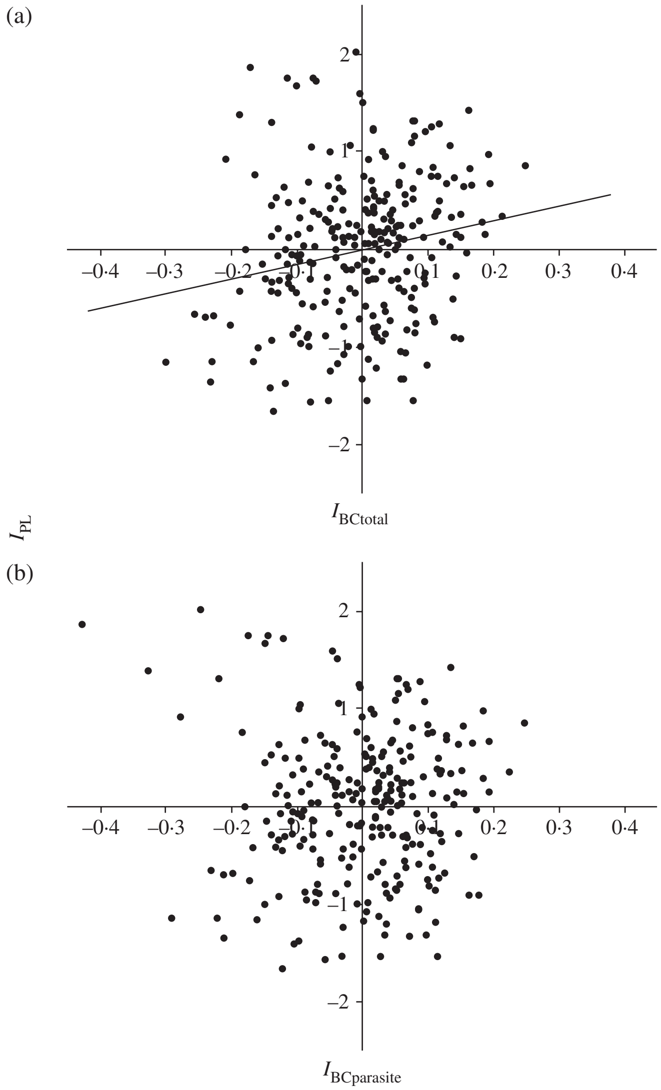

FIG. 3. Regression of parasite load index ($I_{\text{PL}}$) on (a) body condition index of total fish mass (*i.e.* recorded mass) minus diet content mass ($I_{\text{BCtotal}}$) of *Gobiomorphus cotidianus* and (b) body condition index of total fish mass minus (diet content mass plus parasite mass) ($I_{\text{BCparasite}}$). Points on the right side of the *y*-axis represent fish in above average condition ($I_{\text{BC}} > 0$), those on the left represent fish in below average condition ($I_{\text{BC}} < 0$). Fish (points) above the *x*-axis carry higher than average parasite load ($I_{\text{PL}} > 0$), and *vice versa* for fish below this axis. (a) The curve was fitted by $y = 1.48100x - 0.00002$.

affecting body condition and survival (Pennycuick, 1971; Crowden & Broom, 1980; Shariff *et al.*, 1980; Karvonen *et al.*, 2004). No such effect was detected in the case of *Tylodelphys* sp. infecting *G. cotidianus*. This lack of pathological effects may be due to the relatively low number (around five per eye) of *Tylodelphys* sp. metacercariae found in *G. cotidianus* compared to studies on *Diplostomum* sp. (Table I; Crowden & Broom, 1980; Karvonen *et al.*, 2004); however, this cannot be determined from the

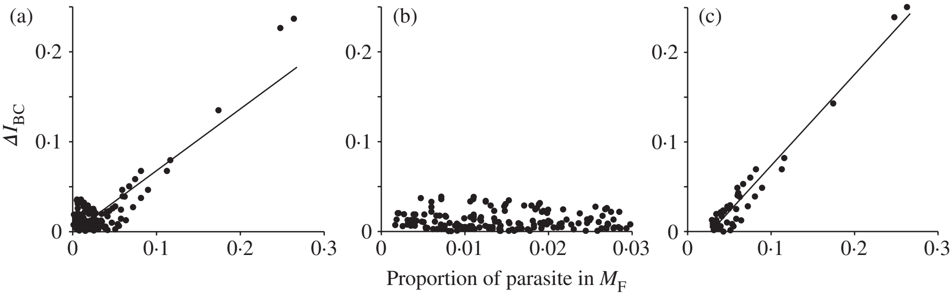

FIG. 4. Regression of the difference between *Gobiomorphus cotidianus* body condition indices ($\Delta I_{BC}$) calculated with ($I_{BC\,total}$) and without ($I_{BC\,parasite}$) parasite mass estimated for each individual ($\Delta I_{BC} = I_{BC\,total} - I_{BC\,parasite}$) on the proportion of parasite tissue in fish mass: (a) overall, (b) in fish containing < 3% of parasite tissue in their body mass and (c) in fish containing > 3% of parasite tissue. The curves were fitted by (a) $y = 0.7301x - 0.0027$ ($r^2 = 0.7062$) and (c) $y = 1.0204x - 0.0290$ ($r^2 = 0.9467$).

present data. Although they were found in almost all fish tissues, the main effects of the other trematode metacercariae infecting *G. cotidianus* probably occur during skin penetration, migration and cyst production within fish tissues. Once metacercariae have established and the cysts are formed, individual parasites probably impose little energetic demands upon the fish (Lemly & Esch, 1984). Some studies suggest that heavy metacercariae loads may affect fish survival, even after penetration and migration, and could be energetically demanding even these effects are not detectable in $I_{BC}$ (Jacobson *et al.*, 2008; Ferguson *et al.*, 2011). Larvae of the nematode *Eustrongylides* sp. were larger but present in low numbers in fish (Table I). Similarly to trematode metacercariae, once they have penetrated the gut wall and encysted, their effect on fish condition is probably limited. Furthermore, infection of *G. cotidianus* by helminth larvae happens gradually over the life time of the fish and not in mass infections, therefore it is possible that these parasites have little effects on fish condition. Juvenile stages of helminths made up most of the parasite mass found in sampled *G. cotidianus* (>97%; Table I); it is thus not surprising that the level of infection (*i.e.* parasite load) had little effect on fish body condition.

Regardless of the actual effects of helminth infection on fish body condition in the present study, the mass of parasites contained within a fish should be taken into account before estimating fish condition using length and mass relationships. This may be particularly important for small-bodied fish species, which tend to harbor a greater mass of parasites per gram of host tissue than larger-bodied fish species (George-Nascimento *et al.*, 2004; Poulin & George-Nascimento, 2007). Considering parasite mass is also especially important when parasite burdens are high and highly variable among conspecific fish. Ignoring its effect on $I_{BC}$ calculation could induce an over estimation of fish condition, increasingly biased towards heavily infected individuals, thus masking actual infection effects on fish condition or inducing spurious correlations between $I_{BC}$ and $I_{PL}$ as observed with the present data. Parasites are contained within the host but parasite tissues are not host tissues and should never be included in the fish mass when estimating its body condition, especially when looking at potential effects of helminth infections of fish condition.

We thank A. Besson, I. Blasco-Costa, M. Warburton and K. Garrett for assistance with field collection and laboratory processing of samples. This study was funded by a grant from the Marsden Fund (New Zealand) to R.P. The study was carried out in accordance with Otago University animal ethics guidelines (Animal Ethics Approval ET 10/12) and under the New Zealand Department of Conservation and Fish and Game fishing permit (NZ – DOC permit OT-34204-RES). The manuscript was significantly improved by very constructive comments from two anonymous reviewers and the assistant editor.

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