



## The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*

A. S. GRUTTER\*‡ AND N. W. PANKHURST†

\*Department of Microbiology and Parasitology, The University of Queensland, Brisbane, Queensland 4072, Australia; and †School of Aquaculture, University of Tasmania, P.O. Box 1214, Launceston, Tasmania 7250, Australia

(Received 29 June 1999, Accepted 25 March 2000)

Tropical labrids *Hemigymnus melapterus* sampled underwater had low plasma levels of cortisol, glucose, and lactate. Plasma cortisol levels were elevated by capture stress within 5–6 min, while glucose and lactate levels were not. Plasma levels of cortisol and glucose increased after 2–4 h of handling and transport to the laboratory. Levels of cortisol and glucose fell with laboratory acclimation back to values similar to those found in wild fish. Parasitism by gnathiid isopods across an order of magnitude of isopod numbers had no effect on plasma levels of cortisol or glucose. Thus, *H. melapterus* has a stress response similar to that shown by temperate species, and relatively high parasite loads are not apparently stressful to fish in the wild. This may be related to the counterproductive effects of physiological stress responses on the immune system or behaviour-modulated processes that counter parasitic invasion.

© 2000 The Fisheries Society of the British Isles

Key words: stress; cortisol; glucose; lactate; gnathiid isopods; labridae; fish; parasites.

### INTRODUCTION

Fish respond to stress with characteristic acute increases in plasma levels of the catecholamines adrenaline and noradrenaline, and slower but more sustained increases in plasma levels of the corticosteroid cortisol (Sumpter, 1997). Increases in catecholamine and corticosteroid levels are generally mirrored by increases in plasma levels of glucose generated by the glucose-mobilizing effects of both classes of hormone (Barton & Iwama, 1991; Wendelaar Bonga, 1997). Also, plasma lactate levels increase typically in stressed fish, particularly if any aspect of the stressor results in increased activity, or a decrease in oxygen availability (Thomas *et al.*, 1999). To date, most of the information on the stress response of fish is derived from studies on salmonids (Sumpter, 1997) and a range of temperate, non-salmonid species (Pankhurst & Sharples, 1992; Vijayan *et al.*, 1993; Sunyer *et al.*, 1995; Waring *et al.*, 1996; Barnett & Pankhurst, 1998). Stress investigations have been conducted also on high latitude species (Ryan, 1995; Lowe & Wells, 1996), but with a few exceptions (Pankhurst *et al.*, 1997) there is very little information on the stress response of tropical species.

Accurate assessment of the stress response is strongly dependent on establishment of resting or basal levels of the physiological parameters used to define the

‡Author to whom correspondence should be addressed. Tel.: 61 7 3365 2471; fax: 61 7 3365 1655; email: a.grutter@mailbox.uq.edu.au

stress response. For domestic stocks, the effectiveness with which this can be done appears to be dependent mainly on the quality of husbandry (Wendelaar Bonga, 1997), whereas for wild fishes the single most important factor is to sample fish as soon after initial disturbance as possible. One of the most effective ways of achieving this is by capturing fish underwater and taking a blood sample *in situ* (Pankhurst & Sharples, 1992).

In the present study, the stress response of the tropical labrid *Hemigymnus melapterus* (Bloch) to capture, handling, and confinement stress was investigated in fish that were captured and sampled first underwater. *Hemigymnus melapterus* was chosen for the study as it is a common species on shallow coral reefs throughout the Indo-Pacific, and because it is the focus of companion studies examining the effects of cleaner fish activity on loads of parasitic gnathiid isopods on labrid hosts (Grutter, 1999a). In association with variable access to cleaner fish, caged *H. melapterus* show substantial differences in gnathiid loads (Grutter, 1999a). Gnathiids can be deleterious to fish: when abundant they inflame and destroy mucosal tissues and cause bacterial infections in stingrays *Dasyatis* spp. (Honma & Chiba, 1991) and can kill captive fish (Paperna & Por, 1977; Mugridge & Stallybrass, 1983). This study investigated whether the high incidence of parasites on some individuals was stressful to fish, and whether one of the benefits of cleaning might be the reduction of physiological stress. To address these questions, plasma levels of cortisol, glucose and lactate were measured in *H. melapterus* sampled immediately after underwater capture, after handling for 2–4 h, and after 2.5 months of confinement in the laboratory. Parasite loads of fish at capture were measured to assess the effect of differential levels of infestation with gnathiid isopods on physiological stress status.

## MATERIALS AND METHODS

### FISH CAPTURE

Fish were collected between 14 December 1997 and 2 February 1998, from seven sites on the shallow reef flat (1.5 m) surrounding Lizard Island and two nearby islands, Palfrey and South Island on the Cairns sector of Australia's Great Barrier Reef. Fish were captured by herding against a 15 × 1.6 m barrier net and captured with a hand-net at the net face. Fish were placed in plastic bags to reduce loss of gnathiids due to handling (Grutter, 1995), then bled immediately underwater ( $n=39$ ), using the technique described in Pankhurst (1990). Blood samples were all taken within 6 min of capture (87% were captured within  $\leq 4$  min). Live fish and plastic bag contents were placed in separate 10-l buckets with aeration for transport by boat (10–30 min) to the Australian Museum's Lizard Island Research Station. Fish were held in the shade for 2–3 h prior to removal of parasitic gnathiid isopods. To achieve this, fish were anaesthetized in MS-222 ( $0.1 \text{ g l}^{-1}$ ) for 3–5 min, their gills, fins, and body surfaces scraped gently with the tip of a wash bottle, and then fish were soaked in a formalin/freshwater bath ( $0.25 \text{ ml l}^{-1}$ ) for 3–5 min to remove any remaining gnathiids. This method recovers 98% of gnathiids (Grutter, 1999b). Some fish were bled again (see below) upon return to the laboratory. All fish held in the laboratory were supplied with running water from the sea at ambient temperature (28–30°C). Fish were fed daily *ad libitum* with chopped prawns and ate readily from the second day of capture. Fish weighed between 40 and 333 g.

### EFFECTS OF HANDLING STRESS

The short-term effects of handling (1–6 min) were examined in fish sampled underwater ( $n=39$ ) by recording the interval between the time when fish were captured and the time

they were sampled. The effect of capture and handling on cortisol and glucose (there was insufficient plasma for lactate) was examined in six fish, captured as before, then held in 10-l buckets for 2–4 h before being bled (before treatment for parasites). The experiment was repeated with five fish, which had been bled previously underwater. The long-term effects of confinement were measured in six fish held in 1000-l tanks for 2.5 months at a density of two fish per tank. One fish per tank was sampled at a time with the second fish sampled 1–3 days later to avoid serial disturbance effects. All captive fish were bled within 4 min of tank disturbance.

#### PLASMA ANALYSES

Blood samples were placed on ice immediately after sampling except for underwater samples which were placed on ice after the dive (usually within 1 h of sampling). Blood samples were centrifuged, the plasma separated and frozen and stored at  $-20^{\circ}\text{C}$ . Plasma cortisol levels were determined by radioimmunoassay in samples shipped frozen to the University of Tasmania, using the reagents and protocols described in Pankhurst & Sharples (1992) and Pankhurst & Carragher (1992) respectively. Plasma levels of glucose and lactate were measured using standard spectrophotometric assays (15-UV and Sigma enzymatic kits 826-UV respectively).

#### STATISTICAL ANALYSES

The effects of handling on plasma cortisol, glucose, and lactate levels were analysed by one way ANOVA and Tukey's means comparison tests. To examine the effect of gnathiids on cortisol and glucose (lactate was not included as there was insufficient plasma to measure lactate from all fish sampled underwater), separate multiple regressions were performed, with the blood parameter as the dependent variable and gnathiid abundance and fish standard length as the independent variables. Fish standard length was included as gnathiid abundance is known to increase with fish size (Grutter & Poulin, 1998). To meet the assumptions of homogeneity of variance in the ANOVAs and regressions, two outliers with no gnathiids were omitted for the cortisol analyses ( $1.20$  and  $1.66\text{ ng ml}^{-1}$ ), and blood parameters and gnathiid abundance were  $\log_{10}$  transformed.

### RESULTS

Mean plasma cortisol levels in fish sampled underwater were low ( $3\text{ ng ml}^{-1}$ ) but increased markedly to  $40\text{ ng ml}^{-1}$  on exposure to capture, handling, and transporting to the laboratory (Fig. 1). Plasma cortisol levels in fish held in captivity for 2.5 months were elevated ( $11\text{ ng ml}^{-1}$ ) over those of freshly captured fish, but substantially lower than those in acutely stressed fish (Fig. 1). Assessment of fish sampled underwater in relation to the time between capture and subsequent bleeding showed that there was a significant increase in plasma cortisol levels 5–6 min after capture (Fig. 2). However, most fish (87%), were captured within  $\leq 4$  min.

Plasma glucose levels in fish sampled underwater were  $\sim 2\text{ mmol l}^{-1}$ , and rose significantly to  $\sim 4\text{ mmol l}^{-1}$  following acute stress from handling (Fig. 1). After 2.5 months of captivity, plasma glucose levels had fallen to  $<0.5\text{ mmol l}^{-1}$  (Fig. 1). In contrast to cortisol, plasma glucose levels did not vary in relation to the time between capture and subsequent bleeding ( $P>0.05$ ) (Fig. 3).

Mean levels of plasma lactate in fish sampled underwater were low ( $\sim 1.5\text{ mmol l}^{-1}$ ) and declined to  $<0.5\text{ mmol l}^{-1}$  in fish held in captivity for 2.5 months (Fig. 1). Similar to glucose, plasma lactate levels did not vary in relation to the time between capture and subsequent bleeding (2 min from

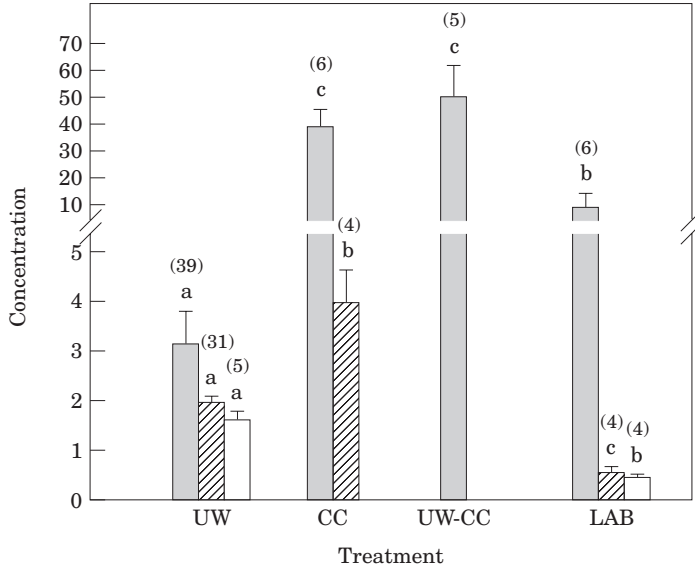


FIG. 1. Plasma levels (mean  $\pm$  S.E.) of cortisol ( $\text{ng ml}^{-1}$ ) ( $\square$ ), glucose ( $\text{mmol l}^{-1}$ ) ( $\text{hatched}$ ), and lactate ( $\text{mmol l}^{-1}$ ) ( $\square$ ) in fish rapidly captured and sampled underwater (UW), sampled after capture and bucket confinement (CC), sampled after underwater bleeding and bucket confinement (UW-CC), or sampled after 2.5 months in the laboratory (LAB). Values with different superscripts are significantly different ( $P < 0.05$ ). Samples sizes are in brackets.

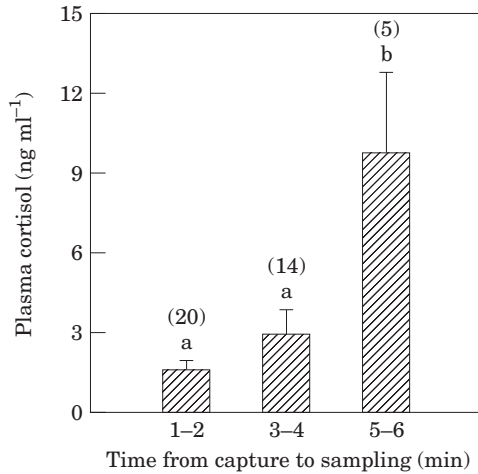


FIG. 2. Plasma cortisol levels ( $\text{ng ml}^{-1}$ , mean  $\pm$  S.E.) in fish captured underwater in relation to the interval between capture and blood sampling. Values with different superscripts are significantly different ( $P < 0.05$ ). Sample sizes are in brackets.

capture:  $1.35 \text{ mmol l}^{-1}$  S.E.  $0.244$ ,  $n=2$ ; 5-6 min from capture:  $1.892 \text{ mmol l}^{-1}$  S.E.  $0.341$ ,  $n=3$ ,  $P > 0.05$ ). Gnathiid loads per fish ranged from 0-27 across all fish sizes, and varied by approximately one order of magnitude for a particular fish size. There was no relationship between plasma cortisol or glucose levels, and parasitic gnathiid loads, even after accounting for fish size (Figs 4 and 5).

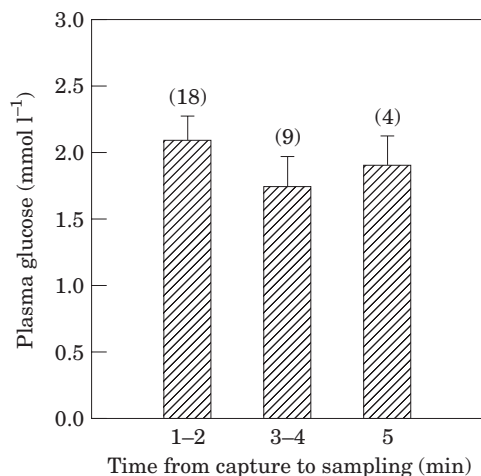


FIG. 3. Plasma glucose levels ( $\text{mmol l}^{-1}$ , mean  $\pm$  S.E.) in fish captured underwater in relation to the interval between capture and blood sampling. Sample sizes are in brackets.

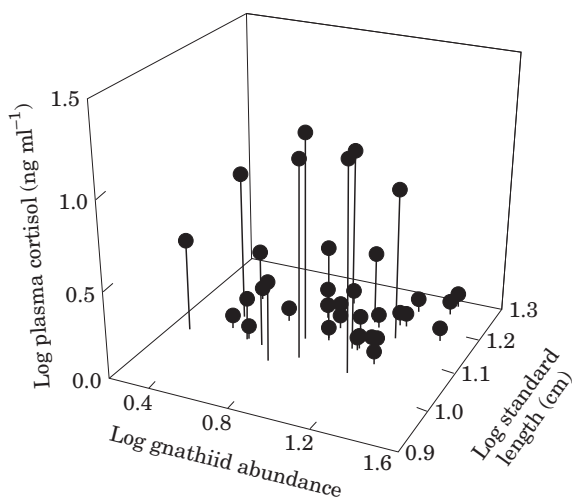


FIG. 4. Plasma cortisol levels per fish of fish captured and sampled underwater rapidly compared with parasitic gnathiid isopod abundance and fish standard length. A multiple regression with log plasma cortisol as the dependent variable and  $\log_{10}$  gnathiid abundance and  $\log_{10}$  standard length as the independent variables, was not significant ( $P > 0.05$ ).

## DISCUSSION

The low basal cortisol levels ( $3 \text{ ng ml}^{-1}$ ) found in *H. melapterus* at capture from the reef were consistent with values from individuals of other species sampled from the wild with small delay (typically  $< 10 \text{ ng ml}^{-1}$ ; Pankhurst & Sharples, 1992), and domestic stocks maintained under conditions of good husbandry and low disturbance (typically  $< 5 \text{ ng ml}^{-1}$ ; Sumpter, 1997). This adds further support to the view that studies reporting substantially higher cortisol levels in wild fish reflect excessive delay between capture and sampling,

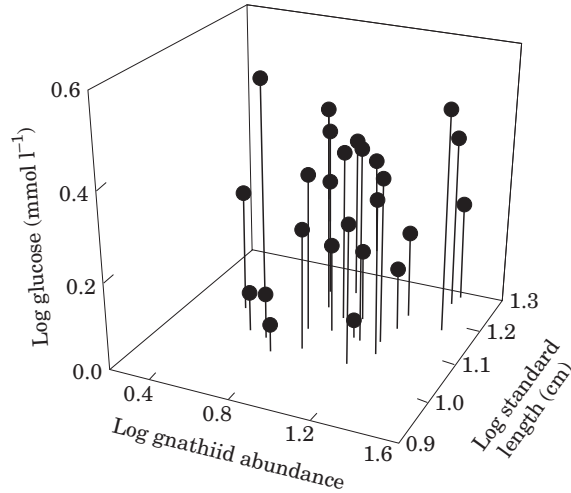


FIG. 5. Plasma glucose levels per fish of fish captured and sampled underwater rapidly compared with parasitic gnathiid isopod abundance and fish standard length. A multiple regression with log plasma cortisol as the dependent variable and  $\log_{10}$  gnathiid abundance and  $\log_{10}$  standard length as the independent variables, was not significant ( $P > 0.05$ ).

and are not representative of resting or basal cortisol levels (Pankhurst & Sharples, 1992). That basal cortisol levels are of similar magnitude in freshwater salmonids (Sumpter, 1997), temperate marine fishes (Pankhurst & Sharples, 1992), Antarctic fishes (Ryan, 1995) and tropical fishes (Pankhurst *et al.*, 1997; this study) suggests that corticosteroid physiology under normal conditions is very similar across a wide range of phylogenies and environments.

*Hemigymnus melapterus*, which were sampled at 28–30° C, showed a rapid increase in plasma cortisol in response to capture stress, with significant elevations occurring as early as 5 min after first disturbance. This is towards the shorter end of the window of response latency reported for other species, but similar to temperate blue mao mao *Scorpiis violaceus* (Hutton) at 13–15° C (Pankhurst *et al.*, 1992), cold temperate salmonids at 4–10° C (Sumpter *et al.*, 1986) and warm temperate red drum *Scianops ocellatus* (L.) at 20–26° C (Robertson *et al.*, 1988). This suggests that the response latency is relatively hard wired and not strongly influenced by temperature. An exception to this may be the Antarctic nototheniid *Pagothenia borchgrevinki* (Boulenger), where response latencies may be as long as 1 h (Ryan, 1995). This suggests that the capacity to compensate for temperature may be impaired at the very low Antarctic sea temperatures (–1.9° C). Elevated plasma cortisol is a consistent response to capture, handling, and confinement; however, there are differential increases across species, probably relating both to phylogenetic differences, and differential experimental protocols used in the various studies (Barton & Iwama, 1991). *Hemigymnus melapterus* showed mean peak plasma levels of cortisol of *c.* 50 ng ml<sup>-1</sup> which is toward the lower end of values shown by other species (Barton & Iwama, 1991; Pankhurst & Sharples, 1992). It remains to be demonstrated whether this reflects use of a more mild stressor in the present than in some of the other studies, or is a species characteristic.

*Hemigymnus melapterus* showed some recovery of plasma cortisol levels in the laboratory after 2.5 months, albeit to levels that were still elevated over those found in freshly captured fish. This appears to be a consistent response of wild fish held in captivity (Pankhurst, 1998) and suggests that wild fish may never acclimate completely to the conditions of captivity. This view is supported by the finding that stress responses are more severe in wild than in domestic stocks of the same species (Woodward & Strange, 1987), and that stress responses in wild salmonids remain unchanged even after long periods of captivity (Salonius & Iwama, 1993).

Plasma lactate levels were low in *H. melapterus* at capture, and still low after laboratory acclimation, and similar to basal values reported for other species (Booth *et al.*, 1995; Barnett & Pankhurst, 1998). The fall in plasma lactate levels seen in laboratory acclimated fish suggests that struggling during capture in the wild may already have elevated lactate levels a little above normal. Small plasma volumes in samples from acutely stressed fish precluded measurement of lactate levels under these conditions; however, plasma lactate levels remained unchanged in caged fish that showed some high cortisol levels individually, suggesting that elevated plasma lactate levels are not a requisite component of the stress response in *H. melapterus*. Part of this may be explained by the behavioural response of *H. melapterus* to disturbance. Startled fish are briefly active and then commonly assume an immobile position in the tank or the cage. This may mean that unlike active species such as salmonids (Thomas *et al.*, 1999), stressed *H. melapterus* typically do not enter anaerobiosis.

The absence of acute increases in plasma lactate levels at sample times when cortisol levels did increase suggests that either the conditions of capture and sampling did not induce anaerobiosis, or that lactate accumulation did occur but without significant release of lactate ions to the plasma. Given the generally low levels of lactate recorded throughout, the former seems likely. Plasma glucose levels in *H. melapterus* at capture were lower than those reported for laboratory acclimated northern pike *Esox lucius* L. (~4 mM; Schwalm & Mackay, 1985), domestic rainbow trout *Oncorhynchus mykiss* (Walbaum) (~4.5 mM; Wells & Pankhurst, 1999), and laboratory acclimated golden perch *Macquaria ambigua* Richardson (~4 mM; Braley & Anderson, 1992), but similar to laboratory acclimated sea raven *Hemitripterus americanus* Gmelin (~1 mM; Vijayan & Moon, 1994).

Stress elevated plasma glucose to levels similar to those found in stressed sea ravens (~4 mM) but considerably lower than those in stressed trout, pike or golden perch (10–18 mM, references as above). The smaller increases in plasma glucose with stress in *H. melapterus* may, as discussed earlier, reflect the relatively low severity of the stressor used, or it may indicate that there is only a modest capacity for stress-induced increases in plasma glucose in this species.

Plasma glucose levels fell to lower levels in laboratory acclimated *H. melapterus*. This may indicate that basal plasma glucose levels are <1 mM, in which case the proportional increase in plasma glucose levels following stress is as large as in other species. As glucose mobilization appears to be controlled primarily by catecholamines, and as rises in plasma catecholamine levels almost certainly would have occurred already at sampling after wild capture (Wenderlaar Bonga, 1997), it is quite possible that the plasma glucose levels

recorded from wild fish sampled underwater in the present study, were already showing stress-induced changes. However, the fact that there was no evidence of an acute increase in plasma glucose level with time taken to sample suggests that this may not be the case.

Most studies examining the role of stress and parasites have involved administration of exogenous cortisol and the subsequent effect on the host's susceptibility to parasites (Pickering & Duston, 1983; Woo *et al.*, 1987; Johnson & Albright, 1992) or its effect on the stress response of parasitized fish (Ruane *et al.*, 1999). This study, in contrast, examined whether parasites are a stressor and thus whether their abundance is correlated to levels of cortisol.

The lack of correlation of plasma levels of either cortisol or glucose with gnathiid isopod load over an order of magnitude difference in the level of infestation (for the same sized fish) suggests that ectoparasites do not act as a stressor in *H. melapterus*. Sampling events do not explain these patterns as most fish (87%) were sampled within 4 min. The gnathiids involved were relatively small (usually <1.5 mm) compared to gnathiids that harm hosts (Paperna & Por, 1977; Honma *et al.*, 1991). Also they remain on the host for only minutes or hours (A. S. Grutter, unpubl. data). However, *Hemigymnus melapterus* are attacked constantly by gnathiids, both day and night (Grutter, 1999b). Furthermore, laboratory experiments show that *H. melapterus* show a behavioural response to gnathiids, with those parasitized with gnathiids seeking cleaners, while those without do not (A. S. Grutter, unpubl. data). As cleaner fish remove gnathiids from *H. melapterus* at such a rapid rate that gnathiid abundance drops daily (Grutter, 1999a), their abundance on fish rarely may have reached high enough levels to elicit a stress response. This suggests parasites cause a degree of irritation without eliciting a stress response. Alternatively, repeated short-term infestation may constitute a chronic stress. Under some conditions fish respond to chronic stress with desensitization of the HPI axis (Sumpter, 1997).

There is some evidence from other species to support the contention that parasites do not generate typical stress responses. Rainbow trout infected with the haemoflagellate *Cryptobia salmositica* Katz showed no changes in plasma levels of glucose or cortisol, despite evidence of pathological changes (Laidley *et al.*, 1988). Similarly, cortisol and glucose levels were unchanged in rainbow trout infected with a pathogenic fungus (Rand & Cone, 1990), and these authors concluded that activation of the hypothalamic-pituitary-interrenal axis was not a normal component of the disease response. Nolan *et al.* (1999) reported changes in epithelial structure of skin and gills, and elevations of serum chloride and the activity of gill Na<sup>+</sup>/K<sup>+</sup> ATPase in Atlantic salmon smolts *Salmo salar* L. exposed to controlled numbers of caligid copepods, and concluded that this was indicative of a stress response. However, the study did not assess whether the hypothalamo-pituitary-interrenal axis was activated as a result of infestation.

Chronic artificial or stress-induced elevation in plasma cortisol suppresses the immune response of fishes (Balm, 1997), and greatly reduces the capacity of fish to deal with infection challenge (Pickering & Duston, 1983; Woo *et al.*, 1987; Pickering & Pottinger, 1989; Johnson & Albright, 1992). Because of this, it is probably adaptively inappropriate for sustained disease or parasitic challenge to stimulate a classical stress response. Elevated plasma corticosteroid levels result also in behavioural changes in a range of vertebrates including fish (Pankhurst

*et al.*, 1999). Because access to cleaner fish requires maintenance of certain behavioural characteristics, such as posturing behaviour (Potts, 1973), elevated cortisol levels and the associated potential for behaviour modification may again be counterproductive in terms of parasite removal.

This study was funded by an Australian Research Council Postdoctoral Fellowship and Small Grant awarded to ASG, and Australian Research Council Infrastructure and Large Grants awarded to NWP. Thanks are extended to M. Johnson for help in the field and to P. Hilder for assistance with plasma analyses.

## References

- Balm, P. H. M. (1997). Immune-endocrine interactions. In *Fish Stress and Health in Aquaculture* (Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B., eds), pp. 195–221. Cambridge: Cambridge University Press.
- Barnett, C. W. & Pankhurst, N. W. (1998). The effects of common laboratory and husbandry practices on the stress responses of greenback flounder *Rhombosolea tapirina* (Günther, 1862). *Aquaculture* **162**, 313–329.
- Barton, B. A. & Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects on corticosteroids. *Annual Review of Fish Diseases* **1**, 3–26.
- Booth, R. K., Kieffer, J. D., Davidson, K., Bielak, A. T. & Tufts, B. L. (1995). Effects of late-season catch and release angling on anaerobic metabolism, acid-base status, survival, and gamete viability in wild Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 283–290.
- Braley, H. & Anderson, T. A. (1992). Changes in blood metabolite concentration in response to repeated capture, anaesthetic and blood sampling in the golden perch, *Macquaria ambigua*. *Comparative Biochemistry and Physiology* **103A**, 445–450.
- Gutter, A. S. (1995). A comparison of methods for sampling ectoparasites of coral reef fishes. *Marine and Freshwater Research* **46**, 897–903.
- Gutter, A. S. (1999a). Cleaner fish really do clean. *Nature* **398**, 672–673.
- Gutter, A. S. (1999b). Infestation dynamics of parasitic gnathiid isopod juveniles on the coral reef fish *Hemigymnus melapterus* (Labridae). *Marine Biology* **135**, 545–552.
- Gutter, A. S. & Poulin, R. (1998). Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. *Marine Ecology Progress Series* **164**, 263–271.
- Honma, Y. & Chiba, A. (1991). Pathological changes in the branchial chamber wall of Stingrays, *Dasyatis* spp., associated with presence of juvenile gnathiids Isopoda, Crustacea). *Gyobyo Kenkyu* **26**, 9–16.
- Honma, Y., Tsunaki, S., Chiba, A. & Ho, J. (1991). Histopathological studies on the juvenile gnathiid (Isopoda, crustacea) parasitic on the branchial chamber wall of the stingray, *Dasyatis akajei*, in the Sea of Japan. *Reports of the Sado Marine Biological Station, Niigata University* **21**, 37–47.
- Johnson, S. C. & Albright, L. J. (1992). Effects of cortisol implants on the susceptibility and the histopathology of the responses of naïve coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14**, 195–205.
- Laidley, C. W., Woo, P. T. K. & Leatherland, J. F. (1988). The stress response of rainbow trout to experimental infection with the blood parasite *Cryptobia salmositica* Katz, 1951. *Journal of Fish Biology* **32**, 253–261.
- Lowe, T. E. & Wells, R. M. G. (1996). Primary and secondary stress responses to line capture in the blue mao mao. *Journal of Fish Biology* **49**, 287–300.
- Mugridge, R. E. R. & Stallybrass, H. G. (1983). A mortality of eels, *Anguilla anguilla* L., attributed to Gnathiidae. *Journal of Fish Diseases* **6**, 81–82.

- Nolan, D. T., Reilly, P. & Wendelaar Bonga, S. E. (1999). Infection with low numbers of sea louse *Lepeophtheirus salmonis* induces stress-related effects in postsmolt Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 947–959.
- Pankhurst, N. W. (1990). Changes in plasma levels of gonadal steroids during spawning behaviour in territorial male demoiselles *Chromis dispilus* (Pisces: Pomacentridae) sampled underwater. *General and Comparative Endocrinology* **79**, 215–225.
- Pankhurst, N. W. (1998). Reproduction. In *Biology of Farmed Fish* (Black, K. D. & Pickering, A. D., eds), pp. 1–26. Sheffield: Sheffield Academic Press.
- Pankhurst, N. W. & Carragher, J. F. (1992). Oocyte maturation and changes in plasma steroid levels in snapper *Pagrus* (= *Chrysophrys*) *auratus* (Sparidae) following treatment with human chorionic gonadotropin. *Aquaculture* **101**, 337–347.
- Pankhurst, N. W. & Sharples, D. F. (1992). Effects of capture and confinement on plasma cortisol concentrations in the snapper *Pagrus auratus*. *Australian Journal of Marine and Freshwater Research* **43**, 345–356.
- Pankhurst, N. W., Wells, R. M. G. & Carragher, J. F. (1992). Effects of stress on plasma cortisol levels and blood viscosity in blue mao mao, *Scorpius violaceus* (Hutton), a marine teleost. *Comparative Biochemistry and Physiology* **101A**, 335–339.
- Pankhurst, N. W., Barnett, C. W., Butler, P. I., Pankhurst, P. M. & Hobby, A. C. (1997). Environmental disturbance, reproductive behaviour and plasma steroid levels in the spiny damselfish *Acanthochromis polyacanthus*. In *Advances in Comparative Endocrinology* (Kawashima, S. & Kikuyama, S., eds), pp. 1707–1713. Bologna: Monduzzi Editore.
- Pankhurst, N. W., Hilder, P. I. & Pankhurst, P. M. (1999). Reproductive condition and behavior in relation to plasma levels of gonadal steroids in the spiny damselfish *Acanthochromis polyacanthus*. *General and Comparative Endocrinology* **115**, 53–69.
- Paperna, I. & Por, F. D. (1977). Preliminary data on the Gnathiidae (Isopoda) of the northern Red Sea, the Bitter Lakes and the eastern Mediterranean and the biology of *Gnathia piscivora* n. sp. *Rapports de la Commission Internationale pour la Mer Méditerranée* **24**, 195–197.
- Pickering, A. D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *Journal of Fish Biology* **23**, 163–175.
- Pickering, A. D. & Pottinger, T. G. (1989). Stress response and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry* **7**, 253–258.
- Potts, G. W. (1973). The ethology of *Labroides dimidiatus* (Cuv. & Val.) (Labridae, Pisces) on Aldabra. *Animal Behaviour* **21**, 250–291.
- Rand, T. G. & Cone, D. K. (1990). Effects of *Ichthyophonus hoferi* on condition indices and blood chemistry of experimentally infected rainbow trout (*Oncorhynchus mykiss*). *Journal of Wildlife Diseases* **26**, 323–328.
- Robertson, L., Thomas, P. & Arnold, C. R. (1988). Plasma cortisol and secondary stress responses of cultured red drum (*Scianops ocellatus*) to several transportation procedures. *Aquaculture* **68**, 115–130.
- Ruane, N. M., Nolan, D. T., Rotlant, J., Tort, L., Balm, P. H. M. & Wendelaar Bonga, S. M. (1999). Modulation of the response of rainbow trout (*Oncorhynchus mykiss*) to confinement, by an ectoparasitic (*Argulus foliaceus* L.) infestation and cortisol feeding. *Fish Physiology and Biochemistry* **20**, 43–51.
- Ryan, S. N. (1995). The effect of chronic heat stress on cortisol levels in the Antarctic fish *Pagothenia borchgrevinki*. *Experientia* **51**, 768–774.
- Salonius, K. & Iwama, G. K. (1993). Effects of early rearing environment on stress response, immune function and disease resistance in juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 759–766.
- Schwalme, K. & Mackay, W. C. (1985). The influence of exercise-handling stress on blood lactate, acid-base and plasma glucose status of northern pike (*Esox lucius* L.). *Canadian Journal of Zoology* **63**, 1125–1129.

- Sumpter, J. P. (1997). The endocrinology of stress. In *Fish Stress and Health in Aquaculture* (Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B., eds), pp. 95–118. Cambridge: Cambridge University Press.
- Sumpter, J. P., Dye, H. M. & Benfey, T. J. (1986). The effects of stress on plasma ACTH,  $\alpha$ -MSH and cortisol levels in salmonid fishes. *General and Comparative Endocrinology* **62**, 377–385.
- Sunyer, J. O., Gómez, E., Navarro, V., Quesada, J. & Tort, L. (1995). Physiological responses and depression of humoral components of the immune system in gilthead sea bream (*Sparus aurata*) following daily acute stress. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 2339–2346.
- Thomas, P. M., Pankhurst, N. W. & Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *Journal of Fish Biology* **54**, 1177–1196.
- Vijayan, M. M. & Moon, T. W. (1994). The stress response and plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Canadian Journal of Zoology* **72**, 379–386.
- Vijayan, M. M., Foster, G. D. & Moon, T. W. (1993). Effects of cortisol on hepatic carbohydrate metabolism and responsiveness to hormones in the sea raven, *Hemirhamphus americanus*. *Fish Physiology and Biochemistry* **12**, 327–335.
- Waring, C. P., Stagg, R. M. & Poxton, M. G. (1996). Physiological responses to handling in the turbot. *Journal of Fish Biology* **48**, 161–173.
- Wells, R. M. G. & Pankhurst, N. W. (1999). Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *Journal of the World Aquaculture Society* **30**, 276–284.
- Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews* **77**, 591–625.
- Woo, P. T. K., Leatherland, J. F. & Lee, M. S. (1987). *Cryptobia salmositica*: cortisol increases the susceptibility of *salmo gairdneri* Richardson to experimental cryptobiosis. *Journal of Fish Diseases* **10**, 75–83.
- Woodward, C. C. & Strange, R. J. (1987). Physiological stress responses in wild and hatchery-reared rainbow trout. *Transactions of the American Fisheries Society* **116**, 574–579.