Journal of Fish Biology (2009) **74,** 1525–1531
doi:10.1111/j.1095-8649.2009.02218.x, available online at http://www.blackwell-synergy.com

Tethering induces increased stress artifacts in social fish species

A. R. Pursche*†‡, I. M. Suthers† and M. D. Taylor*†

Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Science, University of New South Wales, Sydney 2052, Australia and †Sydney Institute of Marine Science, Bldg 2, Chowder Bay Road, Mosman, New South Wales 2088, Australia

(Received 11 May 2008, Accepted 29 January 2009)

Behaviour of juvenile mulloway *Argyrosomus japonicus* were investigated under laboratory conditions to determine the efficacy of estimating predation mortality using tethering. The occurrence and duration of stressed behaviour was evaluated for individual *A. japonicus* that were hooked but untethered, hooked and tethered and unhooked and untethered (free swimming), both in schools and in isolation. Tethered and hooked treatments showed a significantly higher incidence and duration of stressed behaviour over controls, but stressed behaviour was lower for hooked but untethered fish in the presence of a school. Artifacts associated with elevated stress may reduce the reliability of estimates of relative predation derived from tethering data for schooling fishes.

Key words: *Argyrosomus japonicus*; behaviour stress; mortality; mulloway; tethering.

INTRODUCTION

Tethering is commonly used for estimating relative predation pressure by testing for effects of small-scale variation in habitat (Nakamura & Sano, 2004), predator abundance (Kellison *et al.*, 2002), tide (Clark *et al.*, 2003), depth (Manderson *et al.*, 2004), turbidity (Gregory & Levings, 1998) and diel variability (Clark *et al.*, 2003). Small-scale effects on predation can be tested by evaluating presence, absence or injury to a fish tethered to a mooring after a period of time. Tethering allows the placement of a potential prey species into defined regions where the relative effect of exogenous processes on predation mortality can be assessed.

Several experimental artifacts of tethering have been recognized (Peterson & Black, 1994) and discussed at length (Adams *et al.*, 2004), however, they are yet to be thoroughly tested and quantified. Given the potential level of stress and discomfort caused to the prey item by the attachment device, and associated ethical concerns, a strong emphasis should be placed on the quality of

‡Author to whom correspondence should be addressed. Tel.: +61 2 93852118; fax: +61 2 93851558; email: a.pursche@student.unsw.edu.au

information obtained using this approach. The ability of tethering-derived data to reflect actual effects on predation needs to be considered in terms of anti-predator and other social behaviours displayed by the target species. A review of five recent studies on mobile prey species shows that behavioural artifacts are rarely addressed in the experimental design (Table I).

The present study aims to demonstrate the importance of social behaviour in the use and interpretation of mortality estimates from tethering experiments. Specifically, juvenile mulloway *Argyrosomus japonicus* (Temmink & Schlegel) (Sciaenidae) were tethered under controlled conditions to determine relative rates of stress behaviour associated with attachment of tethering apparatus to a solitary *A. japonicus* and a single *A. japonicus* within a school. *Argyrosomus japonicus* are a schooling demersal fish occurring in estuarine and coastal waters of eastern Australia (Gray & McDonall, 1993). *Argyrosomus japonicus* stock enhancement trials for this species are currently underway (Taylor *et al.*, 2006), and tethering has been proposed as a method of evaluating the effect of different stocking techniques (*i.e.* time, size and habitat of release) on post release mortality.

MATERIALS AND METHODS

Experiments were carried out in 10 000 l tanks (1·65 m radius) on a flow-through aquarium system at Port Stephens Fisheries Centre (NSW Department of Primary Industries, Australia). Project aims were assessed by trialling three types of tethering device (hooked and tethered, hooked but not tethered and not tethered or hooked), in the presence and absence of other conspecifics in a factorial design with 15 replicates per treatment combination.

All *A. japonicus* (51·07 ± 1·19 mm L_T; mean ± S.E.) were anaesthetized for 30 s using 5 mg l^{-1} aqui-s (AQUI-S; www.aqui-s.com) prior to the three treatments. Two factors tested were: (a) attachment (three-levels: hook only, hook and tether and control or no attachment) and (b) school (two-levels: one fish tethered in isolation and one fish tethered within a school). In the first treatment of factor (a), the effect of the hook only on the behaviour of *A. japonicus* was examined by placing a size 12 hook through the surface skin of the ventral side of the caudal peduncle but leaving the hook unattached to the mooring. There was no blood observed at the point of attachment. In the second treatment of factor (a), the effect of a complete tethering device was examined by inserting the hook as described above and fastening the hook to a mooring *via* a 1 m piece of 1·82 kg test nylon line and a brass swivel, which had a combined mass of <0·01 g. Previous studies have varied tether lengths and deployment durations from 200 to 500 mm and between 15 min and 24 h, respectively (Danilowicz & Sale, 1999; Haplin, 2000; Linehan *et al.*, 2001; Laurel *et al.*, 2003; Adams *et al.*, 2004). Moorings consisted of a half-brick attached to a float by 6 mm rope with the tether attached to the base of the line. The third treatment of factor (a) was a procedural control for the anaesthesia, and fish were anaesthetized as described above before being placed back into the treatment tank. The experimental subject was placed in isolation or in the presence of 30 conspecifics factor, (b). In the schooling treatments, conspecifics were added to the tank 30 min prior to addition of the tethered or control fish. A sample mooring was present within the tank for all control and untethered treatments. All fish were euthanized using a lethal dose of benzocaine at the completion of the experiment in accordance with acceptable procedures of finfishes (Barker *et al.*, 2002).

Behaviour of the subject fish was observed for 20 min, with behaviour noted as stressed or calm (Fielder & Bardsley, 1999). Stressed behaviour included swimming irregularly (darting), inverting or twisting and were defined as either continuous (>3 s) or instantaneous (<3 s). Calm behaviour was defined as swimming slowly but

TABLE I. Review of five tethering experiments using schooling species and recognition of behavioural artefacts

Species	Prey response?	Does the prey school?	Is tethered behaviour considered?	Is schooling behaviour considered?	Did artifacts override experiment?	Reference
Cod *Gadus* spp.	Flight	Yes	Yes	No	No	Laurel *et al.* (2003)
Cod *Gadus* spp.	Flight	Yes	Yes	No	No	Linehan *et al.* (2001)
Surgeonfish *Acanthurus* sp.	Flight	Yes	Yes	No	Yes	Adams *et al.* (2004)
French grunt *Haemulon* sp.	Flight	Yes	Yes	No	No	Danilowicz & Sale (1999)
Mummichog *Fundulus* sp.	Flight	Yes	Yes	No	No	Haplin (2000)

constantly, maintaining their position within the water column or schooling with conspecifics when present. Behaviour was quantified as the frequency of stress or calm behaviour across each 2 min time period, and data were standardized to 'stressed behaviour' min^{-1}. Variance of standardized behavioural data was homogenous for continuous ($Q = 0{\cdot}19$) and instantaneous ($Q = 0{\cdot}15$) behaviours using Cochran's test and was compared between treatments using a two-factor ANOVA in Minitab v. 14 (www.minitab.com).

RESULTS

Behavioural results pooled across time showed significant effect on the frequency of continuous stress behaviours by treatment type ($F_{2,45}$, $P < 0{\cdot}05$) and the presence of a school ($F_{2,45}$, $P < 0{\cdot}05$), with the tether treatment resulting in a higher rate of stress than the control [Bonferroni, $P = 0{\cdot}01$; Fig. 1(a)].

There was a significant difference in instantaneous stress behaviours between tethering treatments [$F_{2,45}$, $P < 0{\cdot}01$; Fig. 1(b)], with stress significantly higher in the tethering treatment relative to the control (Bonferroni, $P < 0{\cdot}01$) and hook (Bonferroni, $P < 0{\cdot}01$) treatments. A significant interaction term ($F_{2,45}$, $P < 0{\cdot}01$) was subjectively interpreted as a higher level of stress in schooling fish than solitary fish for tether treatments but no difference for the hook and the control [Fig. 1(b)]. Instantaneous behaviour was not affected by the presence of a school for any treatment.

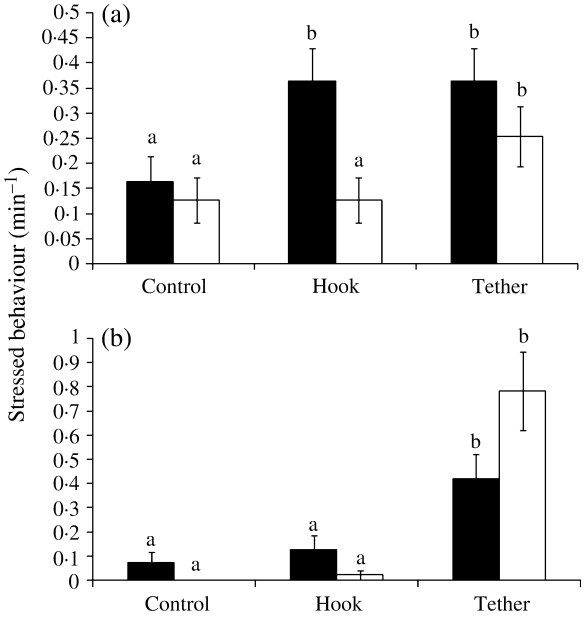

FIG. 1. *Argyrosomus japonicus* mean ± S.E. (a) continuous or (b) instantaneous stress behaviours in hooked, tethered and non tethered, single (■) or schooling (□) treatments. Similar lower-case letters (a, a) between bars indicate non-significant differences. Alternate lower-case letters (a, b) between bars indicates significant differences.

DISCUSSION

This study provides the first examination under controlled conditions, of tether-induced stress responses by schooling fish. Tethered *A. japonicus* exhibited increased instantaneous and continuous distress behaviours when attached to both a hook, and a complete tethering apparatus and stress behaviours were significantly higher in the absence of a school. Increased stress has been shown to increase susceptibility to predation in juvenile coho salmon *Oncorhynchus kisutch* (Walbaum) (Olla & Davis, 1992). The higher degree of movement and stress observed under these conditions may provide additional stimulus to olfaction predators, which increases susceptibility to predation by attracting predators to tethered prey. This may produce artificially high rates of predation that mask the relative effects of exogenous processes an experiment is designed to detect.

Tethering of slow, benthic invertebrates such as prawns (Haywood & Pendrey, 1996) has been considered acceptable as the potential prey item is either slow moving and cannot reach the end of the tether (Shears & Babcock, 2002) or behaves in a way as not to reach the end of the tether by burrowing within the sediment (Kellison *et al.*, 2003). Tethering of more mobile species such as fishes and large prawns, however, may not provide an accurate indication of mortality. Previous studies have recognized adverse prey behaviour from tethers as a potential artefact influencing predation (Danilowicz & Sale, 1999), and predation upon tethered prey has been shown to be nine times greater than that of untethered prey (Kneib & Scheele, 2000). While there is potential for experimental artifacts when tethering (Aronson & Heck, 1995; Aronson *et al.*, 2001; Adams *et al.*, 2004), interference of the tethering device with normal behaviour is rarely quantified. Similarly, comparisons of predation on tethered and untethered prey items have failed to account for behavioural effects of the tether on solitary social fish species.

Estimating natural mortality of any species in open systems has long been a challenge for fisheries scientists (Pauly, 1980). Techniques exist for assessing long-term natural mortality (Ricker, 1958; Pauly, 1980), but few field options are available for estimating short-term geocentric mortality. Inherent experimental artifacts of tethering are well known (Haywood *et al.*, 2003), and the technique provides a crude estimation of relative mortality at best. Tethering may remain useful for comparing relative mortality between environmental variables, however, suitability of the technique is species specific. Tethering of an entire school of prey-fishes to overcome isolation-induced stress is impractical in the field, and the strength of stress behaviours in tethered, schooling fish species may overshadow subtle effects between habitats on predation. Also, the increased stress levels experienced during tethering of *A. japonicus* and associated artifacts in the data obtained may preclude ethical justification for the technique, considering the discomfort that is probably experienced by the experimental fish.

Given the behavioural observations presented here, *A. japonicus* are not suitable for tethering experiments, and the issues identified above are probably applicable to tethering other Sciaenidae and schooling species. Future field studies should be coupled with laboratory experiments to ensure that results

can be interpreted in the context of inherent behavioural artifacts of tethering experiments.

We would like to thank S. Fielder, H. El Hassan and staff at Port Stephens Fisheries Centre for provision of facilities and assistance throughout the experiment. We also wish to thank the editor of the Journal of Fish Biology and the two anonymous reviewers of this manuscript for their thoughtful input into our work. The investigation was authorized by the University of New South Wales Animal Care and Ethics Committee (permit 06/42A) and funded by the New South Wales Recreational Saltwater Fishing Trust.

References

Adams, A. J., Miller, M. E. & Ebersole, J. P. (2004). Tethers make juvenile surgeonfish (Acanthuridae) vulnerable to attacks by benthic invertebrates. *Bulletin of Marine Science* **74,** 207–211.

Aronson, R. B. & Heck, K. L. (1995). Tethering experiments and hypothesis testing in ecology. *Marine Ecology Progress Series* **121,** 307–309.

Aronson, R. B., Heck, K. L. & Valentine, J. F. (2001). Measuring predation with tethering experiments. *Marine Ecology Progress Series* **214,** 311–312.

Clark, K. L., Gregory, M. R. & Hines, A. H. (2003). Diel variation in predator abundance, predation risk and prey distribution in shallow-water estuarine habitats. *Journal of Experimental Marine Biology and Ecology* **287,** 37–55.

Danilowicz, B. S. & Sale, P. F. (1999). Relative intensity of predation on the French grunt, *Haemulon flavolineatum*, during diurnal, dusk, and nocturnal periods on a coral reef. *Marine Biology* **133,** 337–343.

Fielder, D. S. & Bardsley, W. (1999). A preliminary study on the effects of salinity on the growth and survival of mulloway *Argyrosomus japonicus* larvae and juveniles. *Journal of the World Aquaculture Society* **30,** 380–387.

Gray, C. A. & McDonall, V. C. (1993). Distribution and growth of juvenile mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae), in the Hawkesbury River, south-eastern Australia. *Australian Journal of Marine and Freshwater Research* **44,** 401–409.

Gregory, R. S. & Levings, C. D. (1998). Turbidity reduces predation on migrating juvenile pacific salmon. *Transactions of the American Fisheries Society* **127,** 275–285.

Haplin, P. M. (2000). Habitat use by an intertidal salt-marsh fish: trade-offs between predation and growth. *Marine Ecology Progress Series* **198,** 203–214.

Haywood, M. D. E. & Pendrey, R. C. (1996). A new design for a submersible chronographic tethering device to record predation in different habitats. *Marine Ecology Progress Series* **143,** 307–312.

Haywood, M. D. E., Manson, F. J., Loneragan, N. R. & Toscas, P. J. (2003). Investigation of artifacts from chronographic tethering experiments – interactions between tethers and predators. *Journal of Experimental Marine Biology and Ecology* **290,** 271–292.

Kellison, G. T., Eggleston, D. B. & Tanaka, M. (2002). Density-dependent predation and implications for stock enhancement with Japanese flounder. *Journal of Fish Biology* **60,** 968–980.

Kellison, G. T., Eggleston, D. B., Taylor, J. C., Burke, J. S. & Osborne, J. A. (2003). Pilot evaluation of summer flounder stock enhancement potential using experimental ecology. *Marine Ecology Progress Series* **250,** 263–278.

Kneib, R. T. & Scheele, C. E. H. (2000). Does tethering of mobile prey measure relative predation potential? An empirical test using mummichogs and grass shrimp. *Marine Ecology Progress Series* **198,** 181–190.

Laurel, B. J., Gregory, R. S. & Brown, J. A. (2003). Predator distribution and habitat patch area determine predation rates on age-0 juvenile cod *Gadus* spp. *Marine Ecology Progress Series* **251,** 245–254.

Linehan, J. E., Gregory, R. S. & Schneider, D. C. (2001). Predation risk of age-0 cod (*Gadus*) relative to depth and substrate in coastal waters. *Journal of Experimental Marine Biology and Ecology* **263,** 25–44.

Manderson, J. P., Pessutti, J., Hilbert, J. G. & Juanes, F. (2004). Shallow water predation risk for a juvenile flatfish (winter flounder; *Pseudopleuronectes americanus*, Walbaum) in a northwest Atlantic estuary. *Journal of Experimental Marine Biology and Ecology* **304,** 137–157.

Nakamura, Y. & Sano, M. (2004). Is there really lower predation risk for juvenile fishes in a seagrass bed compared with an adjacent coral area? *Bulletin of Marine Science* **74,** 477–482.

Olla, B. L. & Davis, M. W. (1992). Comparison of predator avoidance capabilities with corticosteroid levels induced by stress in juvenile coho Salmon. *Transactions of the American Fisheries Society* **121,** 544–547.

Pauly, D. (1980). Interrelationships between natural mortality growth parameters and mean environmental temperature in 175 fish stocks. *Journal du Conseil international pour l'Exploration de la Mer* **39,** 175–192.

Peterson, C. H. & Black, R. (1994). An experimentalists challenge: when artifacts of intervention interact with treatments. *Marine Ecology Progress Series* **111,** 289–297.

Ricker, W. E. (1958). Handbook of computation for biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* **119,** 1–300.

Shears, N. T. & Babcock, R. C. (2002). Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia* **132,** 131–142.

Taylor, M. D., Laffan, S. D., Fielder, D. S. & Suthers, I. M. (2006). Key habitat and home range of mulloway *Argyrosomus japonicus* in a south-east Australian estuary: finding the estuarine niche to optimise stocking. *Marine Ecology Progress Series* **328,** 237–247.

Electronic Reference

Barker, D., Allen, G. L., Rowland, S. J. & Pickles, J. M. (2002). A guide to acceptable procedures and practices for aquaculture and fisheries research. In *NSW Fisheries Animal Care and Ethics Committee Report*. Nelson Bay, NSW: Port Stephens Fisheries Centre. Available at http://www.dpi.nsw.gov.au/fisheries/aquaculture/publications/general

