

Original article

Net-chasing training improves the behavioral characteristics of fish and crustaceans

Kohji Takahashi ^{1,*} and Reiji Masuda ²¹ Nagasaki University, Nagasaki, Nagasaki, 852-8521, Japan² Kyoto University, Maizuru, Kyoto, 625-0086, Japan

* Correspondence: ta.kohji@gmail.com; Tel.: +81-90-9342-9993

Keywords: Conditioning; Learning; Stress training; Stock enhancement.

Received: 18 July 2017 / Accepted: 3 September 2017

© 2017 by the authors.

Introduction

Stock enhancement is a proactive fisheries management tool for the recovery of decreasing fisheries resources that primarily uses hatchery-reared seedlings [1]. However, these seedlings often possess behavioral characteristics that are not adaptable to the release environment [2] due to them being reared in a safe and comfortable tank environment. Therefore, the rearing environment of seedlings needs to be altered to improve their adaptability.

Net-chasing in a rearing tank is one approach that has been used to train fishes avoidance responses to quasi-predator attacks and threatening situations. However, although it has been reported that repeated net-chasing in a rearing tank changes the behavioral characteristics of some fishes [3, 4], few studies have evaluated whether net-chasing can be used as a training method for the released fish. Furthermore, no studies to date have investigated whether net-chasing can also be used to change the behaviors of invertebrate species. Therefore, here, we investigated the effect of net-chasing on the behavioral characteristics of the fishes Japanese flounder (*Paralichthys olivaceus*) and red sea bream (*Pagrus major*) and the crustacean Indian bait prawn (*Palaemon pacificus*).

Materials and methods

Paralichthys olivaceus

To investigate whether a net-chasing treatment would improve the off-bottom swimming behavior of *P. olivaceus* during foraging, we chased the juveniles with a hand net for 1 minute two to four times per day for 2 weeks. We then compared their behavior with those of control fish that had never been exposed to the net-chasing.

We observed fish for 3 min when feeding from the surface on the day immediately prior to the first treatment and 2 weeks after the final treatment. During this time, we measured the frequency and duration of

off-bottom swimming. Analysis of variance (ANOVA) was then used to compare the off-bottom behavior pre- and post-treatment within and between treatments.

Pagrus major

To investigate whether a net-chasing treatment would improve the behavioral characteristics of *P. major*. We chased the fish for 2 min twice daily for 3 weeks, following which the fish were provided with 1 week of conventional rearing prior to behavioral testing. We then compared their behavior with those of control fish that had been reared in a conventional manner for 4 weeks with no net-chasing treatment.

We conducted three consecutive behavioral tests: an emergence test, an avoidance test, and a foraging test. The emergence test evaluated the boldness of fish [3]. A fish was introduced to the start area of the experimental tank, and recording the time taken for the fish to emerge from this area during 3600 s. A survival analysis was then used to compare the emergence rate of the net-chased and control groups. In the avoidance test, we evaluated the alertness of fish by measuring their avoidance of the safety area in the experimental tank when presented with a novel object (a small hand net). We then used a Fisher's exact test to compare the avoidance rates between treatments. In the foraging test, we evaluated stress recovery by individually transferring each fish into an experimental tank, providing pellets 30 and 90 min after transfer, and observing their foraging behavior. We then used a Fisher's exact test to compare the ratio of foraging fish at 30 and 90 min between treatment groups.

Once the behavioral tests had been completed, we conducted a predator exposure test. For each trial, we simultaneously placed three net-chased and three control fish into the experimental tank and then introduced a predator (marbled rockfish, *Sebastes marmoratus*) 10 min later. The trial was terminated when only three of the individuals were confirmed to be alive. We then analyzed the survival rate of fish using a Wald test with a generalized linear mixed model

(GLMM) that included “treatment”, “fin clipping”, “elapsed days” and “standard length (SL)” as fixed effects. We also calculated the odds ratio of the total number of surviving fish in each treatment to evaluate the effect of the net-chasing treatment.

Palaemon pacificus

To investigate whether a net-chasing treatment could also change the behavioral characteristics of a crustacean species, we chased Indian bait prawn for 2 min twice per day for 8 days and then measured two behaviors (sheltering and avoidance) on the day after the final treatment. In the sheltering behavior test, a prawn was introduced to the experimental tank and 5 min later an artificial weed was provided as shelter. We then measured the time taken by the prawn to take shelter and the duration of sheltering over a 5-min period. In the avoidance test, we observed the avoidance response of each prawn when it was touched by a pipette. We analyzed the sheltering behavior and avoidance rate of prawns using a likelihood ratio test with a GLMM that included “treatment” and “body length (BL)” as fixed effects.

Results and discussion

Paralichthys olivaceus

There was no significant difference in the total frequency and duration of off-bottom swimming pre- and post-treatment in the net-chased group ($P > 0.05$), whereas there was a significant increase in both of these post-treatment in the control group ($P < 0.01$). Furthermore, the frequency of off-bottom swimming post-treatment was significantly lower in the net-chased group than in the control group ($P < 0.05$). These results suggest that the control fish increased the frequency and duration of off-bottom swimming during the course of the experiment, and that net-chasing can be used to prevent such an increase.

Pagrus major

In the emergence test, the net-chased group of *P. major* took significantly less time to emerge than the control group ($P < 0.01$), which indicates that *P. major* that are trained by net chasing can quickly return to their regular behavior in a novel environment. Furthermore, in the avoidance test, significantly more fish escaped in the net-chased group than in the control group ($P < 0.05$), which suggests that the fish had attained the ability to avoid a dangerous situation by experiencing daily threats. In the foraging test, there was no significant difference in foraging rate between the treatment groups after 30 min ($P > 0.05$), but the foraging rate was significantly higher in the net-chased group than in the control group after 90 min ($P < 0.05$), suggesting that net-chased fish attain a resistance to the stress that is induced by environmental change.

In the predator exposure test, “treatment” and “SL” were found to have significant effects on survival (treatment: $P < 0.005$; SL: $P < 0.05$), demonstrating that net-chased fish and larger fish had a higher survival rate than control fish and smaller fish, respectively. Therefore, since there was no significant difference in size between the treatment groups, the survival rate of net-chased fish will have been enhanced by the net-chasing treatment. Furthermore, the odds ratio for fish survival in the net-chased treatment group was +6.76 against control fish, indicating that net-chasing enhanced the anti-predator behavior of *P. major*.

Palaemon pacificus

We found that the net-chased prawns entered the shelter significantly more quickly than the control prawns ($P < 0.05$) and also had a significantly higher avoidance response rate than the control prawns ($P < 0.05$). These results indicate that a net-chasing treatment will not only enhance the behavior of fishes, but will also increase the alertness of prawns.

Conclusions

We found that the use of a net-chasing treatment successfully improved the behavioral characteristics that are useful for survival in a natural environment in both *P. olivaceus* and *P. major*. Thus, this treatment may have a wider applicability for training seedlings prior to their release to improve their survival than previous training methods, such as learning specific predator information. Furthermore, we also demonstrated that net-chasing can be used to improve the behavioral characteristics of Indian bait prawn, indicating that this treatment would also be useful for training the seedlings of invertebrate species. Since net-chasing can be used to enhance the survival skills of released fish, fewer fish would need to be released, which, in return, would reduce any potential interference by stock enhancement. Further studies are now required to develop this training method so that it can be applied the conventional rearing of seedlings and to verify its effects in a natural environment.

Acknowledgements

The present study was funded by the Grant-in-Aid for JSPS Fellows (JSPS KAKENHI Grant Number JP8), and the Sasakawa Scientific Research Grant from The Japan Science Society. We thank Dr. Takeshi Takegaki to support our study of Indian bait prawn.

References

1. Molony BW et al. (2003) *Rev Fish Biol Fish* 13: 409–432
2. Brown C, Day RL (2002) *Fish Fish* 3: 79–94
3. Brown C et al. (2007) *Behav Ecol Sociobiol* 62: 237–243
4. Smith BR, Blumstein DT (2012) *Behaviour* 149: 187–213