Fish Sci (2013) 79:157–162
DOI 10.1007/s12562-012-0592-3

ORIGINAL ARTICLE

Fisheries

Stress hormone responses in ayu *Plecoglossus altivelis* in reaction to different catching methods: comparisons between electrofishing and cast netting

Satoshi Awata · Tetsuya Tsuruta · Takashi Yada · Kei'ichiro Iguchi

Received: 14 August 2012 / Accepted: 25 December 2012 / Published online: 25 January 2013

Abstract It is important to select the most appropriate method for fish sampling in streams and rivers; the sampling efficiency as well as any negative effect of the method of fish sampling on fishes such as stress, injury, and mortality must be considered. This study aimed to investigate stress hormone responses in ayu *Plecoglossus altivelis* in reaction to direct current (DC) electrofishing, alternating current (AC) electrofishing, and cast netting in artificial streams. The mortality, injury rate, and catch efficiency of these catching methods were also compared. It was found that immediately after capture, fish caught using a cast net exhibited significantly higher cortisol levels than did control fish that were caught rapidly by a hand net. Cortisol levels did not differ between electroshocked fish and the controls. Time required to catch 12 fish was shorter when DC was used (20 s) than when AC was used (45 s). The time required to catch fish greatly increased when a cast net was used (840 s). Cortisol levels in DC electroshocked and control treatment groups were at resting levels 24 and 48 h after capture. However, higher cortisol levels were found in fish captured using a cast net at both these time points. Cortisol levels in AC electroshocked fish returned to lower levels at 24 h, but increased again at 48 h. Furthermore, 48 h following capture, the fish caught by AC electrofishing exhibited higher mortality (7.5 %) than those captured by other methods (0 %). Spinal injury was also detected in one of the fish in this group. Considering these findings, DC electrofishing is the most effective and least damaging method for collecting *P. altivelis* in streams with respect to stress, physical damage, and efficiency.

Keywords Cortisol · Catching efficiency · DC electrofishing · AC electrofishing · Cast net · *Plecoglossus altivelis*

Introduction

To collect fishes for research purposes from rivers and streams, various sampling techniques are used such as cast netting, line fishing and traps. In addition to these methods, electrofishing has become a popular technique due to its convenience and efficiency [1–4]. However, many studies have demonstrated that electroshock treatment has a detrimental effect on fishes (e.g. [5, 6]). Electroshocking often causes spinal damage, reduced growth, and increased mortality in fishes, particularly salmonid fishes (e.g. reviewed

S. Awata · T. Tsuruta · K. Iguchi
Ueda Station, Freshwater Fisheries Research Division, National Research Institute of Fisheries Science, Fisheries Research Agency, 1088 Komaki, Ueda 386-0031, Japan

Present Address:
S. Awata (✉)
Sado Marine Biological Station, Faculty of Science, Niigata University, 87 Tassha, Sado 952-2135, Japan
e-mail: sa-awata@cc.niigata-u.ac.jp; satoshijulido@yahoo.co.jp

T. Tsuruta
Department of Human Life and Environment, Faculty of Human Environment, Osaka Sangyo University, 3-1-1 Nakagaito, Daito 574-8530, Japan

T. Yada
Nikko Station, Freshwater Fisheries Research Division, National Research Institute of Aquaculture, Fisheries Research Agency, 2482-3 Chugushi, Nikko 321-1661, Japan

K. Iguchi
Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

in [5, 7]). These studies have also found that mortality and injury rates differ considerably depending on the type of current [continuous direct current (DC), pulsed DC, or alternating current (AC)], wave form, voltage, and duration of shock (salmonids: [8–13]; white sturgeon: [14]; Atlantic herring: [15]). Pulsed DC or AC electrofishing is more damaging than DC electrofishing [6, 8]. Hence, many authors suggest investigating the use of electrofishing to catch freshwater fish to be of high precedence (e.g. [6, 8, 14, 15]). Quantifying the damage resulting due to different fishing techniques, including electrofishing, would be invaluable in gauging the benefits from the viewpoint of animal welfare.

When capturing fishes, researchers should also be concerned about the effects of capture on the physiological stress response in fish. Stress, via the action of cortisol, may result in indirect mortality of fish due to deterioration of immune function (e.g. [16–18]). Similarly, freshwater fishes that have encountered stressors often suffer from increased susceptibility to diseases [19]. Despite extensive research on the effects of electrofishing on physical damage and fish mortality [5], the stress response to electrofishing has been less studied. Consistent results show that regardless of fish species, physiological indicators of stress such as plasma cortisol, glucose, and lactic acid markedly increase after electroshocking (e.g. [20–24]). However, these studies have not compared fish stress responses resulting due to DC and AC electrofishing. Taking into account studies on the negative effects of electricity on fish and the injuries and physiological stress induced by the fishing technique, electrofishing seems to be an improper method for catching freshwater fishes. However, these studies have seldom addressed whether electrofishing causes more physical damage or induces more severe stress responses than any other available sampling methods used to collect freshwater fishes (but see [25]). Irrespective of the method utilized, capture and handling will induce stress in fish. In addition, improper capturing and/or poor handling will often cause physical damage to the fish. Considering these facts, only the most efficient, least stressful, and least damaging method for fish capture should be employed.

The aim of our study was to investigate the stress hormone response of the ayu *Plecoglossus altivelis* to different fishing methods. *P. altivelis* is a typical amphidromous fish in East Asia that spends most of its life in freshwater rivers or streams. It is also a commercially important species for freshwater fisheries [26–28]. Net casting and electrofishing have been widely used as catching methods by researchers who have studied the ecology and aquaculture of *P. altivelis* (electrofisher: e.g. [29–31]; cast net: e.g. [32–34]). However, no studies have investigated the effect of cast netting and electrofishing on the physiological responses, physical damage, and mortality in *P. altivelis*. Therefore, we assessed the stress hormone responses of *P. altivelis* to different catching methods (DC electrofishing, AC electrofishing, and net casting) by measuring the changes in serum cortisol concentrations at 0, 24, and 48 h after capture. We included 24 and 48 h time periods to evaluate both acute and chronic damage to *P. altivelis*. The catch efficiency, injury rates, and 48-h mortality rates observed in the different catching techniques were also compared. From these results, we show the most effective and the least damaging method for collecting *P. altivelis* in streams with respect to stress, physical damage, and efficiency.

Materials and methods

Experimental procedure

Young *P. altivelis* captured in a river discharging into Lake Biwa were obtained from a trader. The fish were reared from May to July 2009 in two outdoor stock ponds [0.9 × 4.2 × 0.9 m (width × length × depth), 0.6 m in water depth] at the Ueda Station, National Research Institute of Fisheries Science, Fisheries Research Agency, Ueda, Japan. Each pond was continuously supplied with water from a natural stream through an inlet. Fish density was approximately 220 fish/m^3 (500 fish per pond) or 4.6 kg/m^3. During this rearing period, the fish were maintained under natural photoperiod at 15.2–21.5 °C. They were fed three times a day with a commercial diet using automatic feeders until they reached approximately 110 mm in standard length, SL and 17 g in body mass, BM. Note that the breeding season for *P. altivelis* is October–November, and thus all the fish used for these experiments were sexually immature.

Three outdoor artificial streams [2.2 × 4.2 × 0.9 m (width × length × depth), 0.3 m in water depth] adjacent to the stock ponds were used for catching experiments. Water in both the artificial streams and the stock ponds was supplied from the same natural stream, and thus, turbidity, water temperature, and photoperiodicity were identical between the two reservoirs. At 15:30 on the day before catching, about 130 fish were transferred into the three artificial streams (43 ± 3 fish per stream), using a hand net. As a control experiment, 45 fish were transferred into a box-shaped net cage (1.0 × 1.0 × 1.0 m with an open top) set in a pond identical to the stock ponds. Catching experiments were conducted between 11:00 and 13:00 using an electrofisher (Backpack Electrofisher, model 12-B; Smith-Root; http://www.smith-root.com/) or a cast net (Mitani Gyogu; http://www.mitani-gyogu.jp/index.html). Twelve fish in each pond were captured using DC electrofishing (200 V), AC electrofishing (200 V, 45 Hz, 0.3–6 ms), and a cast net (mesh size 17.8 mm, net length 2.8 m, maximum net diameter 4.0 m) by an expert in both electrofishing and net casting (K.I.). These fish were immediately transferred into a lethal dose of anesthesia (200 mg/l of MS-222; Sigma-Aldrich; http://www.sigmaaldrich.com/japan.html), using a hand net, after which their blood was sampled by cutting the caudal peduncle with a small kitchen knife or scalpel. Twelve fish in the net cage for the control experiments were caught using a hand net and immediately administered a lethal dose of anesthesia. Their blood was sampled as described previously, acquired within 30 s from the start of capture to the time when the 12 control fish were administered anesthesia; a previous study has shown that this sampling method does not elevate serum cortisol levels in *P. altivelis* [28]. The fish were then measured (SL, to the nearest 0.1 mm) and weighed (BM, to the nearest 0.1 g). Just before the start of the experiment, the temperature of the water in the reservoirs was 21.5 °C and the turbidity was considerably low, at a value of 3.0 mg/l (measured using a turbidity sensor, TCR-5Z; Kasahara Chemical Instruments Co.; http://www.krkjpn.co.jp/handy/TCR-5Z.htm). Fish densities in the experimental streams and in the net cage were approximately 16 and 45 fish/m^3, respectively, both of which were far lower than those in the stock ponds (220 fish/m^3). Therefore, turbidity and fish density were unlikely to contribute to the stress responses of the fish during experiments (see [19, 28, 35]). In addition, although we were only able to complete one experimental trial for each capture method, we ensured that stream effects were unlikely to be large sources of error by using three identical artificial stream channels.

Catch efficiency by each sampling method was defined as the time from the start of catching to the time when all the 12 fish were administered anesthesia. We did not attempt to catch individual fish, and thus often caught several at once. Three box-shaped net cages, which were identical to the ones used in the control experiments, were prepared in advance to be set in the ponds. The remaining fish in each experimental stream (29 ± 1 fish per stream) were captured using the same catching equipment and immediately transferred into the net cages. Fish in the net cages of control and catching experiments were sampled 24 and 48 h after capturing treatment with a hand net (12 fish per experiment); at this point, their blood samples were collected and body measurements were taken. The sampling method used here was the same as used when the control fish were captured at 0 h after treatment. Wounds, abrasions, and fractures were also checked by observing their bodies. Residual fish in the net cage (alive and dead) were counted to calculate the observed mortality at 48 h. The living fish were then returned to the stock ponds.

Cortisol measurement

Blood was centrifuged at 7,500 rpm for 5 min and the serum was removed and frozen at −30 °C until analysis could be performed. Cortisol measurements were taken on each sample in duplicate using the Cortisol EIA Kit according to the manufacturer's instructions (EA65, Oxford Biomedical Research Inc.; http://www.oxfordbiomed.com/). Further details on cortisol measurement have been described previously [28].

Data analyses

Effect of the sampling methods and time since treatment on serum cortisol concentrations was examined using two-way analysis of variance (ANOVA). Since a significant interaction was detected (see Results), Dunnett's post hoc tests were used to differentiate the cortisol levels between fish captured by DC electrofishing, AC electrofishing, or cast net and control fish at each sampling time. Tukey's honestly significant difference (HSD) post hoc tests were applied when changes in cortisol levels were assessed for each sampling method. To satisfy the normal distribution requirements for parametric tests, outliers with defined values greater or less than 1.5 times the interquartile range were removed prior to analyses. As a consequence, 2 % of the total values were removed. The mean SL and BM of *P. altivelis* were 112.7 mm (range = 71.0–148.0 mm, $n = 129$) and 17.1 g (range = 4.5–41.3 g), respectively. There were no significant differences in SL and BM among the treatment groups (SL: one-way ANOVA, $F_{11,116} = 1.16$, $p = 0.32$; BM: $F_{11,116} = 1.08$, $p = 0.38$). The observed 48-h mortality was compared using Fisher's exact test. All analyses were conducted using SPSS 16.0 (SPSS Inc.; http://www-01.ibm.com/software/analytics/spss/). All given p values are two-tailed and α was set at 0.05.

Results

Significant interactions between sampling methods and time since treatments were detected for cortisol concentrations (two-way ANOVA, $F_{6,117} = 3.92$, $p = 0.001$), indicating that stress hormone responses were different among the sampling methods at the different time points. Therefore, cortisol levels were compared between different sampling methods and control treatments at each sampling time and changes in cortisol levels were assessed for each sampling method.

Immediately after capture, cortisol concentrations were significantly higher in fish caught using a cast net than control fish (Dunnett's post hoc test, $p < 0.0001$; Fig. 1).

The fish caught by DC and AC electrofishing did not have different cortisol concentrations compared to the control fish ($p > 0.10$ for both capture treatment). It took a shorter time to catch 12 fish using DC electrofishing (20 s) compared to AC electrofishing (45 s). More time was required when a cast net was used (840 s). The cortisol concentrations in fish captured by cast net were significantly higher than the levels found in control fish 24 h after capture ($p = 0.04$; Fig. 1). Fish caught by AC electrofishing exhibited greater cortisol levels than the control fish 48 h after capture ($p = 0.05$; Fig. 1). Although not significant, a similar trend was seen in fish captured by cast net at the 48-h time point ($p = 0.07$).

Cortisol levels declined significantly with time following capture treatment in both the DC electrofishing (Tukey HSD post hoc test, 0 vs. 24 or 48 h: $p < 0.02$, 24 vs. 48 h: $p > 0.99$) and cast netting groups (0 vs. 24 or 48 h: $p < 0.0001$, 24 vs. 48 h: $p = 0.58$; Fig. 1). This trend was also observed in fish that received the control treatment (0 vs. 24 h: $p = 0.01$, 0 vs. 48 h: $p = 0.08$, 24 vs. 48 h: $p = 0.69$). Cortisol concentrations after AC electroshocking decreased from 0 to 24 h, but increased again from 24 to 48 h (0 vs. 24 h: $p = 0.0005$, 24 vs. 48 h: $p = 0.02$; 0 vs. 48 h: $p = 0.43$; Fig. 1). The observed 48-h mortality of fish exposed to AC electroshocking (dead/total fish = 3/40, 7.5 %) was significantly higher than that obtained in fish exposed to both DC electroshocking (0/45, 0 %) and cast netting (0/45, 0 %; Fisher's exact test, $p = 0.03$). Furthermore, one of the 40 fish with AC electroshocking exposure exhibited spinal injury.

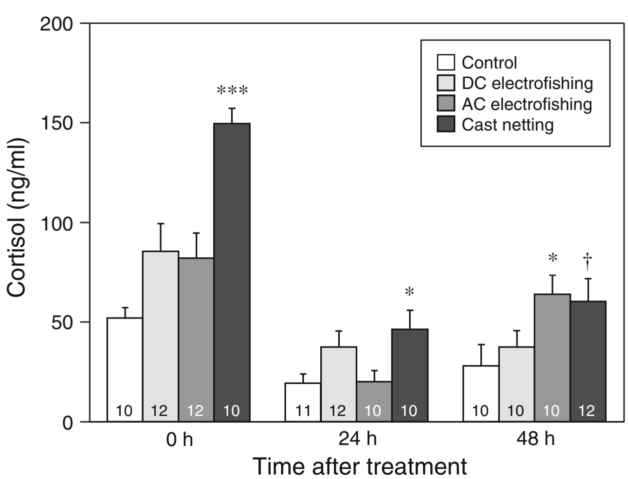

Fig. 1 Serum cortisol concentrations in *Plecoglossus altivelis* 0, 24, and 48 h following DC electrofishing, AC electrofishing, cast netting, or control treatment. *Bars* represent mean + SE. Numerals in *bars* show sample sizes. ***$p < 0.0001$, *$p \leq 0.05$, $^{\dagger}p = 0.07$, compared with control at each sampling time (Dunnett's post hoc test)

Discussion

Numerous studies have documented the negative effects, both physical and physiological, of electrofishing (reviewed in [5]). In addition, many authors have reported the efficiency of electrofishing in comparison to other fish catching methods (e.g. [2–4]). However, based on the reviewed literature, this may be the first study to examine both the harmful effects (stress, injury, and mortality) and the catching efficiency of different catching methods of freshwater fish. Our results found increased cortisol levels in fish caught using a cast net immediately after capture. Cortisol concentrations did not differ between the fish captured by electrofishing (DC and AC) and control fish. Previous studies have also found no significant increase in cortisol levels following exposure to electroshocking ([24, 36, 37]; but see [21]). Elevated cortisol concentrations in fish captured by cast net were probably the result of a continuously stressful situation (840 s needed to catch 12 fish) such as the shadow of a person, a splash produced by the cast net, and/or being netted. In fact, elevations in cortisol levels were generally detected within 10 min after stress exposure (reviewed in [38]). Cast netting in an unconfined natural stream may be less stressful than observed in the artificial streams because *P. altivelis* may be able to move away from and avoid the cast netter. However, such movements would ensure even lower capture efficiency than observed in this study. Nevertheless, this study revealed that the efficiency of electrofishing was found to be higher than that of net casting. Additionally, in the case of *P. altivelis,* electrofishing was less stressful compared to cast netting. Thus, electrofishing is certainly a better method for catching *P. altivelis* in artificial streams, and likely, a preferable technique in natural streams.

Cortisol concentrations significantly decreased in all treatment groups including the controls 24 h after capture. Fish captured by cast net, however, showed higher cortisol levels compared to control fish, and such a trend continued even 48 h after capture. In fish, blood cortisol returns to pre-stress levels within 24 h and generally within 6 h [36, 37, 39, 40]. Similar results were obtained in our studies with *P. altivelis* (Yada T, Awata S, Tsuruta T and Iguchi K, unpubl. data). If the stressful situation continues then the return rates of cortisol may be slowed [38]. Thus, the relatively high cortisol levels in fish 24 and 48 h after capture might result from other stressors induced during cast netting, such as rubbing of skin mucus and/or scale removal. Average blood cortisol levels in the control fish at 0 h was approximately 50 ng/ml, which is higher than the resting levels previously reported for *P. altivelis* (<10 ng/ml, [28]). This is probably because of the transfer to a new environment a day before the experiment. Similar results were obtained in a previous study [28].

Both control fish and fish captured by DC electrofishing exhibited low levels of blood cortisol 48 h after capture, similar to the levels seen at 24 h. However, cortisol concentrations in fish captured by AC electrofishing were higher than that in control fish at this time. Spinal damage was observed in one fish captured by AC electrofishing but not in the fish captured by DC electroshocking. Although internal examinations using radiography (e.g. reviewed in [5]) were not conducted, several studies have shown that AC electroshocking causes more spinal injuries than do other electrical settings [6, 8, 25]. Thus, the extent of spinal damage after AC electrofishing may be underestimated in our study. Furthermore, in our study, mortality was observed only in the fish captured by AC electrofishing. Taken together, our data comparing three methods of capturing (AC electrofishing, DC electrofishing, and net casting) suggest that AC electrofishing is the most harmful catching method for *P. altivelis*, since it may cause spinal damage resulting in chronic stress and high mortality.

To summarize, our results reveal that in the case of *P. altivelis*, DC electrofishing causes less stress, lower spinal damage, and lower mortality than other methods. Catch efficiency with DC electrofishing was far greater than that of other methods. Therefore, it can be concluded that DC electrofishing is the most effective and the least damaging method for catching *P. altivelis* in artificial streams, and likely in natural streams. Although more research is required to determine whether DC electrofishing is similarly suitable for capturing other freshwater fishes, our findings may be applicable to small fishes occupying a habitat similar to *P. altivelis* (i.e. streams). In fact, many researchers use electrofishing to study the life history, distribution, and movement of freshwater fishes in natural streams (Gobiidae: [41]; Cyprinidae: [42]; Cobitidae: [43]; Salmonidae: [44, 45]) including *P. altivelis* [29, 31]. In addition, as shown in a previous study [37], DC electrofishing may be useful for assessing stress responses in *P. altivelis* since the fishing method does not lead to an immediate increase in cortisol concentrations following catching.

Acknowledgments The authors are thankful to an editor and two anonymous reviewers for their constructive critique of an earlier draft of this paper. This work was supported by research grants from the Fisheries Agency, Japanese Ministry of Agriculture, Forestry, and Fisheries. Care and handling of fish conformed to the guidelines for the use of fishes in research established by the Ichthyological Society of Japan.

References

1. Reynolds JB (1996) Electrofishing. In: Murphy BR, Willis DW (eds) Fisheries techniques, 2nd edn. American Fisheries Society, Bethesda, pp 221–254
2. Pugh LL, Schramm HL Jr (1998) Comparison of electrofishing and hoopnetting in lotic habitats of the lower Mississippi river. N Am J Fish Manag 18:649–656
3. Basler MC, Schramm HL (2006) Evaluation of electrofishing and Fyke netting for collecting black carp in small ponds. Trans Am Fish Soc 135:277–280
4. Poos MS, Mandrak NE, McLaughlin RL (2007) The effectiveness of two common sampling methods for assessing imperilled freshwater fishes. J Fish Biol 70:691–708
5. Snyder DE (2003a) Electrofishing and its harmful effects on fish. Information and Technology Report USGS/BRD/ITR-2003–002, U.S. Government Printing Office, Denver
6. Snyder DE (2003b) Invited overview: conclusions from a review of electrofishing and its harmful effects on fish. Rev Fish Biol Fish 13:445–453
7. Portt CB, Coker GA, Ming DL, Randall RG (2006) A review of fish sampling methods commonly used in Canadian freshwater habitats. Canadian Technical Report of Fisheries and Aquatic Sciences 2604, Ontario
8. Bohlin T, Hamrin S, Heggberget TG, Rasmussen G, Saltveit SJ (1989) Electrofishing—theory and practice with special emphasis on salmonids. Hydrobiologia 173:9–43
9. Sharber NG, Carothers SW, Sharber JP, De Vos JC Jr, House DA (1994) Reducing electrofishing-induced injury of rainbow trout. N Am J Fish Manag 14:340–346
10. Dalbey SR, McMahon TE, Fredenberg W (1996) Effect of electrofishing pulse shape and electrofishing-induced spinal injury on long-term growth and survival of wild rainbow trout. N Am J Fish Manag 16:560–569
11. Ainslie BJ, Post JR, Paul AJ (1998) Effects of pulsed and continuous DC electrofishing on juvenile rainbow trout. N Am J Fish Manag 18:905–918
12. Roth B, Imseland S, Moeller D, Slinde E (2003) Effect of electric field strength and current duration on stunning and injuries in market-sized Atlantic salmon held in seawater. N Am J Aquac 65:8–13
13. Roth B, Moeller D, Slinde E (2004) Ability of electric field strength, frequency, and current duration to stun farmed Atlantic salmon and pollock and relations to observed injuries using sinusoidal and square wave alternating current. N Am J Aquac 66:208–216
14. Holliman MF, Reynolds JB (2002) Electroshock-induced injury in juvenile white sturgeon. N Am J Fish Manag 22:494–499
15. Nordgreen AH, Slinde E, Møller D, Roth B (2008) Effect of various electric field strengths and current durations on stunning and spinal injuries of Atlantic herring. J Aquat Anim Health 20:110–115
16. Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26
17. Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77:591–625
18. Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268
19. Iguchi K, Ogawa K, Nagae M, Ito F (2003) The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). Aquaculture 220:515–523
20. VanderKooi SP, Maule AG, Schreck CB (2001) The effects of electroshock on immune function and disease progression in juvenile spring chinook salmon. Trans Am Fish Soc 130:397–408
21. Cho GK, Heath JW, Heath DD (2002) Electroshocking influences chinook salmon egg survival and juvenile physiology and immunology. Trans Am Fish Soc 131:224–233
22. Bracewell P, Cowx IG, Uglow RF (2004) Effects of handling and electrofishing on plasma glucose and whole blood lactate of *Leuciscus cephalus*. J Fish Biol 64:65–71

23. Barreto RE, Volpato GL (2006) Stress responses of the fish Nile tilapia subjected to electroshock and social stressors. Braz J Med Biol Res 39:1605–1612
24. Barton BA, Dwyer WP (1997) Physiological stress effects of continuous- and pulsed-DC electroshock on juvenile bull trout. J Fish Biol 51:998–1008
25. Hollender BA, Carline RF (1994) Injury to wild brook trout by backpack electrofishing. N Am J Fish Manag 14:643–649
26. Iguchi K, Nishida M (2000) Genetic biogeography among insular populations of the amphidromous fish *Plecoglossus altivelis* as assessed from mitochondrial DNA analysis. Conserv Genet 1:147–156
27. Nelson JS (2006) Family Osmeridae (172)–smelts. In: Nelson JS (ed) Fishes of the world, 4th edn. John Wiley & Sons, Hoboken, pp 194–196
28. Awata S, Tsuruta T, Yada T, Iguchi K (2011) Effects of suspended sediment on cortisol levels in wild and cultured strains of ayu *Plecoglossus altivelis*. Aquaculture 314:115–121
29. Iguchi K, Iwata Y, Nishida M, Otake T (2005) Skip of the routine habitat in an amphidromous migration of ayu. Ichthyol Res 52:98–100
30. Sagawa S, Kayaba Y, Minagawa T, Kawaguchi Y (2006) Catchability of six fish species by electrofishing in an experimental stream. Ecol Civil Eng 8:193–199
31. Shimizu A, Uchida K, Udagawa M, Ohkubo M, Ito H, Yamamoto S, Takasawa T (2008) Multiple spawning of amphidromous type ayu *Plecoglossus altivelis* in a large river, Mogami River system. Fish Sci 74:1283–1289
32. Tago Y, Tsujimoto R (2006) Species, number and size of fishes emerged at the small pool created in a shallow run. Ecol Civil Eng 8:165–178
33. Chang KH, Doi H, Imai H, Gunji F, Nakano S (2008) Longitudinal changes in zooplankton distribution below a reservoir outfall with reference to river planktivory. Limnology 9:125–133
34. Iguchi K (2012) Larger eggs at lower water temperature as a measure to assure effective hatchling size in the landlocked form of ayu, *Plecoglossus altivelis*. Ichthyol Res 59:20–25
35. Awata S, Takeshima H, Tsuruta T, Yada T, Iguchi K (2010) Stress hormone response to long- or short-time exposure to suspended solids in ayu *Plecoglossus altivelis*. Aquac Sci 58:425–427
36. Mesa MG, Schreck CB (1989) Electrofishing mark–recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. Trans Am Fish Soc 118:644–658
37. Maule AG, Mesa MG (1994) Efficacy of electrofishing to assess plasma cortisol concentration in juvenile Chinook salmon passing hydroelectric dams on the Columbia river. N Am J Fish Manag 14:334–339
38. Pankhurst NW (2011) The endocrinology of stress in fish: an environmental perspective. Gen Comp Endocrinol 170:265–275
39. Barton BA (2000) Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. N Am J Aquac 62:12–18
40. Yada T, Azuma T, Hyoudo S, Hirano T, Grau EG, Schreck CB (2007) Differential expression of corticosteroid receptor genes in rainbow trout (*Oncorhynchus mykiss*) immune system in response to acute stress. Can J Fish Aquat Sci 64:1382–1389
41. Ito S, Yanagisawa Y (2006) Determinants of male mating success in a natural population of a stream goby of the genus *Rhinogobius*. J Fish Biol 68:185–195
42. Belica LAT, Rahel FJ (2008) Movements of creek chubs (*Semotilus atromaculatus*) among habitat patches in a plains stream. Ecol Freshw Fish 17:258–272
43. Kawanishi R, Inoue M, Takagi M, Miyake Y, Shimizu T (2011) Habitat factors affecting the distribution and abundance of the spinous loach *Cobitis shikokuensis* in southwestern Japan. Ichthyol Res 58:202–208
44. Dieterman DJ, Hoxmeier RJH (2011) Demography of juvenile and adult brown trout in streams of southeastern Minnesota. Trans Am Fish Soc 140:1642–1656
45. Morita K, Morita SH, Nagasawa T (2011) Seasonal changes in stream salmonid population densities in two tributaries of a boreal river in northern Japan. Ichthyol Res 58:134–142