Bull Environ Contam Toxicol (2012) 89:147–151
DOI 10.1007/s00128-012-0636-5

The Acute and Behavioral Effects of a Copper-Nickel Mixture on Roach *Rutilus rutilus*

Gintaras Svecevičius · Nijolė Kazlauskienė · Edvinas Taujanskis

Received: 12 October 2011 / Accepted: 5 April 2012 / Published online: 20 April 2012

Abstract Semi-static acute toxicity tests were conducted on adult roach, *Rutilus rutilus*, to estimate its sensitivity toward an equitoxic binary mixture (EBM) of copper and nickel. The sum of their individual LC50 values was considered to equal 100 %. The main endpoints of the study were mortality and behavioral responses: detection, locomotor activity, coughing rate and pectoral-fin activity. The 96-h LC50 of EBM was 14.4 (10.1 %–20.5 %), indicating a synergism of individual metals. The most meaningful behavioral results were obtained after 10-min, 1-h and 24-h exposures, and the most sensitive and informative behavioral response was found to be coughing rate. This bioassay response may be used successfully to evaluate wastewaters containing heavy metals for their toxicity toward fish.

Keywords Fish · Roach · Acute toxicity · Behavior · Copper-nickel equitoxic binary mixture · Synergism

Copper and nickel are widely used in various anthropogenic activities, are common persistent water pollutants and are categorized as priority substances (European Parliament and Council Directive 2000/60/EC; US EPA 2006). These metals are included in a list (together with zinc and hexavalent chromium) of selected toxic pollutant-indicators for ambient water quality evaluation and monitoring (SCORECARD 2005).

G. Svecevičius (✉) · N. Kazlauskienė · E. Taujanskis
Laboratory of Hydrobiont Ecology and Physiology, Institute of Ecology, Nature Research Centre, Akademijos 2, 08412 Vilnius-21, Lithuania
e-mail: Gintaras.Svecevicius@takas.lt

Although numerous studies on the toxicity of copper and nickel to fish have been conducted and reviewed over the last decades (see reviews Alabaster and Lloyd 1982; US EPA 1985, 1986, 2007; Eisler 1998a, b), comparatively few studies have been conducted on behavioral responses of fish to the effects of these metals, especially to nickel.

The behavioral responses of fish are important because they are sensitive indicators of the presence of toxicants in ambient water (Atchison et al. 1987; Little and Finger 1990; Scherer 1992; Kane et al. 2005). Behavioral endpoints of fish have been included in the American test battery during standard laboratory toxicity tests to measure the sublethal effects of exposure to chemical substances (ASTM 2008a, b). Furthermore, fish behavioral responses could also be used in bioassay testing (the rapid detection of water toxicity using biological objects and processes) of polluted waters in cases where analytical methods are non-informative because the evaluation of effluent quality by physico-chemical measurements does not provide information regarding the possible synergetic or antagonistic effects that toxicant complexes may have on aquatic life (Flerov 1989). The test-organisms must be widely distributed and readily available; their biology must be well known; their maintenance under laboratory conditions must be simple; they must be sensitive to the polluting substances; and their responses to the toxic effect and its registration must be simple and cost-effective (Flerov 1989).

Usually, the tests are conducted on rainbow trout—a standard species, native to North America and commonly used for aquatic toxicity testing. However, this cold-water rheophilous species needs a number of specific conditions for its maintenance in the laboratory. It is therefore important to find a fish species that is toxicant-sensitive, convenient and a typical resident of the given geographical region.

The roach *Rutilus rutilus* (Linnaeus) could perhaps satisfy the main requirements advanced for bioassay testing because it is one of the most common and widely distributed species in Eurasia. It is found in all kinds of waterbodies (lakes, rivers. channels, brackish estuaries, etc.). The roach are active, quick, frightful but curious fish. To our knowledge, there is no information regarding the behavioral sensitivity of the roach to toxicants. Therefore, our studies with this species as a toxicological test-subject may be of interest.

In the natural environment, fish are exposed to various toxicant mixtures. Therefore, it is very important to evaluate the toxicity of the relevant pollutants in mixture for a better understanding of their joint toxic action. To date, the combined effect of copper and nickel on fish has been poorly investigated.

The objectives of this study were to (1) evaluate the sensitivity of roach to a copper-nickel equitoxic binary mixture by means of acute toxicity tests; (2) estimate the toxic action of a copper and nickel in mixture on roach; (3) determine the sensitivity of roach by means of behavioral tests; (4) perform comparative analysis of the sensitivity of responses studied based on the results of acute lethality and behavioral tests; and (5) evaluate the suitability of roach as a test-subject for the needs of bioassay testing of waters containing heavy metals.

Materials and Methods

Toxicity tests were conducted on adult roach. Test fish were collected from Arinas Lake (Molėtai District, Lithuania). The average total length of test fish was 9.5 ± 0.2 cm and the total average weight was 9.6 ± 0.7 grams (mean ± -SEM, respectively). The fish were acclimatized to laboratory conditions for 1 week prior to testing. They were kept in flow-through 1,000-L holding tanks supplied with continuously-aerated deep-well water (a minimum flow rate of 1 L per 1 gram of their wet body mass per day), under natural illumination and were fed frozen proprietary fish feed (*Chironomus* sp.) daily in the morning; the total amount was no <1 % of their wet body mass per day. The day before and during the tests the fish were not fed.

Deep-well water was used as the dilution water. The average hardness of the water was approximately 284 (271–296) mg/L as $CaCO_3$, the alkalinity was 200 (190–210) mg/L as $CaCO_3$, the pH ranged from 7.9 to 8.1, the temperature was maintained at 14–16°C, the dissolved oxygen concentration was maintained at 8–10 mg/L, and the dissolved organic carbon (DOC) was less than the detection limit (<0.3 mg/L).

Reagent grade copper and nickel sulphates ($CuSO_4·5H_2O$ and $NiSO_4·7H_2O$, 98.5 % purity) («REACHIM» Company, Russia) were used as the toxicants. A stock solution was prepared by dissolving a necessary amount of the heavy metal salts in distilled water.

Toxicity tests were conducted under semi-static conditions with five treatments and one control. Test fish were exposed for 96 h to a series of nominal concentrations of a copper-nickel equitoxic binary mixture (EBM). The exposure concentration range was formed based on 96-h LC50 values of 0.88 mg Cu/L and 48.7 mg Ni/L derived from acute toxicity tests using the same well-water (Svecevicius and Vosyliene 1996; Svecevicius 2010). The sum of these concentrations was accepted as the primary test concentration of the equitoxic copper-nickel mixture and was equated to 100 %. The test concentration range was designed using the dilution factor of 0.5 (100, 50, 25, 12.5 and 6.25 %, respectively). Tanks of 30-L total volume filled to 2/3 with dilution water were used. The water in the tanks was continuously aerated. Seven fish were placed into each tank and acclimatized for 6 h. After that, the necessary amount of stock solution was evenly infused into each tank, the test solutions were renewed every day and the fish transferred into the freshly prepared solutions. Fish mortality observations were made at 24-h intervals.

Fish behavior during the acute toxicity test was evaluated basically according to the method proposed by Svecevičius (2005). Such behavioral responses as latent period of detection response (LPDR) (contact-response duration in seconds), locomotor activity (LA) (5-grade scale ranging from 0 to 4) and coughing rate (CR coughs per min) were recorded. It should be noted that it was impossible to detect and observe gill ventilation frequency (GVF) in roach. However, coughing, a gill-purge reflex was easily observed and registered. Therefore, instead of GVF, pectoral-fin activity (PFA) was recorded as the proposed indicator of pollutant induced stress in fish (Lemly 1983; Tudorache et al. 2009) (counts per min). The detection response was estimated only once at the start of the toxicity test after the fish's first contact with the mixture solution, while other behavioral responses were recorded during 1-min test periods set at 0.17 (10 min), 1, 24, 48, 72 and 96-h intervals, respectively. All test fish behaviors were recorded with a digital PC video camera Sony Handycam HDR-CX305E for further analysis.

Median-Lethal-Concentration (LC50) values and their 0.95 confidence intervals were estimated using the trimmed Spearman-Karber method (Hamilton et al. 1977).

Behavioral data were analyzed statistically through STATISTICA 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA) and GraphPad Instat^{TM} 2.04 (GraphPad Software, San Diego, California, USA). The significance of behavioral responses was established by use of Student's *t*-test (for CR and PFA) and Mann–Whitney's non-parametric *U*-test (for LA) at $p \leq 0.05$.

The dissolved oxygen levels in the tanks, temperature and pH were measured routinely with a hand held multimeter (WTW Multi 340i/SET, Germany). Nominal copper and nickel concentrations were checked during blank tests with an atomic absorption spectrophotometer (SHIMADZU AA-6800, Japan) by flame or graphite furnace techniques using proprietary software. Each sample was analyzed in triplicate. Mean measured concentrations were within 4–6 % of target.

Results and Discussion

The data obtained (Table 1) showed that the exposure duration influenced the LC50 value. The greatest differences were observed between the 24-h and 96-h values, while the 72-h and 96-h values were quite close. The ratio of these latter periods was 1.06, while the ratio between 24-h LC50 and 96-h LC50 was 5.3.

According to the method proposed by Könemann (1981), a Mixture-Toxicity-Index (MTI) was calculated for the 96-h acute toxicity data. Our calculations established that the MTI = 2.80 (>1), indicating a supra-addition of copper and nickel in the tested mixture. This means that the toxicity of the copper-nickel mixture is indicative of a synergism of individual metals.

Behavioral data obtained showed that with an increase in the EBM concentration, the LPDR decreased and amounted to averages of 4.6 ± 0.4, 3.9 ± 0.5, 3.4 ± 0.4 and 2.6 ± 0.5 s (mean ± SEM) at test concentrations of 12.5, 25, 50, and 100 %, respectively. However, at an EBM concentration of 6.25 %, this index became unmeasurable. The intensity of other behavioral responses increased rapidly with an increase in the EBM concentration after 10-min and 1-h exposures, with the exception of the 6.25 % concentration at which a significant decrease in LA was established (Table 2). A highest significant increase in PFA (1.5-fold) was recorded only at an EBM concentration of 100 %. However, with increased exposure duration, PFA significantly decreased after 24 h, then remained unaltered after 48 and 72 h, and thereafter significantly increased at the lowest EBM concentration of 6.25 % after 96-h exposure.

Table 1 Acute toxicity of copper-nickel equitoxic mixture (EBM) on roach

Exposure duration (h)	LC50 (%)	0.95 CI (%)
24	64.0	53.3–76.9
48	32.0	26.6–38.5
72	15.2	10.4–22.3
96	14.4	10.1–20.5

Coughing rate increased consistently and most rapidly. The highest significant increases in CR (16-fold and 24-fold) were recorded at an EBM concentration of 100 % after 1 h and at 50 % after 24 h. Although CR decreased with an increase in exposure duration, it remained at a significant level until the end of the test.

The highest significant increases in LA (5.9-fold and 8.7-fold) were recorded at an EBM concentration of 25 % after 48-h and at 50 % after 24-h exposures. With an increase in exposure duration, LA decreased, reaching control level and significantly decreasing at the end of the test.

It should be noted that with an increase in exposure duration to higher EBM concentrations, the test fish became morbid, demonstrated erratic swimming and sudden changes in LA which caused difficulties in behavioral response measurements. It was evident that the most meaningful behavioral data were obtained after 10-min, 1-h and 24-h exposures and the most sensitive and informative behavioral response was CR.

Such great differences in response sensitivity could be explained, apparently, by their reliance on different categories of behavioral responses as described by Scherer (1992). An increase in CR is intimately associated with respiratory demands and gill irritation or blockage, while changes in PFA and LA can be initiated through chemosensory irritation and may reflect a more non-specific stress response resulting in changes in blood cortisol and glucose levels (Scherer et al. 1986; De Boeck et al. 2001; Gagnon et al. 2006), not inducing any damage in gill structure (Waser et al. 2009).

Acute toxicity data obtained here are in close agreement with the data of other researchers. Alabaster and Lloyd (1982) summarized the data on combined effects of mixtures of toxicants on freshwater fish and concluded that in most cases heavy metals in mixtures are more toxic to fish than separate ones; the toxicity of copper-nickel binary mixtures to the guppy, *Poecilia reticulata* and zebrafish, *Danio rerio*, was more than additive.

Little and Finger (1990) summarized the effects of pollutants on the swimming behavior of fish and concluded that heavy metals at sublethal levels elevate spontaneous swimming activity in fish. The activity increased at 0.006–0.009 mg Cu/L in brook trout *Salvelinus fontinalis* ("safe" concentration 0.0095 mg Cu/L), at 0.05 mg Cu/L in catfish *Arius felis* (72-h LC50 > 0.2 mg Cu/L) and at 0.1 mg Cu/L in largemouth bass *Micropterus salmoides* (48-h LC50 0.96 mg Cu/L). However, contrary to these data, Ellgaard et al. (1995) found hypoactivity in goldfish *Carassius auratus* induced by nickel at concentrations of 25–75 mg/L (96-h LC50 between 75 and 100 mg Ni/L).

Drummond et al. (1973) found that CR gradually increased within 2–24 h in brook trout exposed to sublethal

Table 2 Behavioral responses (mean ± SEM) of roach exposed to a copper-nickel equitoxic binary mixture (EBM)

EBM concentration (%)	Exposure duration (h)					
	0.17(10 min)	1	24	48	72	96
Pectoral-fin activity (PFA) (counts/min)						
0 (control)	51.3 ± 3.1 (7)		57.4 ± 3.1 (7)	60.4 ± 3.1 (7)	64.7 ± 4.0 (7)	58.1 ± 2.8 (7)
6.25	50.1 ± 3.1 (7)	52.1 ± 3.0 (7)	58.0 ± 7.6 (7)	59.4 ± 4.7 (7)	60.0 ± 6.4 (6)	78.7 ± 4.8* (6)
12.5	54.6 ± 3.7 (7)	55.9 ± 5.0 (7)	71.7 ± 6.1 (7)	60.4 ± 5.5 (7)	59.4 ± 12.0 (5)	66.4 ± 6.7 (5)
25	60.3 ± 9.1 (7)	57.0 ± 5.6 (7)	63.2 ± 7.6 (7)	Immeasurable (6)	Immeasurable (1)	–
50	59.1 ± 5.0 (7)	59.7 ± 5.1 (7)	30.8 ± 7.7 (6)*	–	–	–
100	74.6 ± 6.4 (7)*	74.7 ± 5.8 (7)*	–	–	–	–
Coughing rate (CR) (counts/min)						
0 (control)	1.3 ± 0.4 (7)		1.9 ± 0.3 (7)	2.3 ± 0.3 (7)	0.9 ± 0.3 (7)	4.9 ± 1.1 (7)
6.25	2.3 ± 0. 5 (7)*	5.1 ± 0.8 (7)*	5.7 ± 1.7 (7)*	4.6 ± 1.0 (7)*	4.0 ± 0.7 (6)*	4.8 ± 1.5 (6)
12.5	4.9 ± 1.3 (7)*	5.3 ± 0.9 (7*)	9.2 ± 1.3 (7)*	7.2 ± 1.5 (7)*	5.6 ± 1.2 (5)*	8.6 ± 0.9 (5)*
25	5.4 ± 1.0 (7)*	6.4 ± 1.6 (7)*	20.2 ± 2.7 (7)*	Immeasurable (6)	Immeasurable (1)	–
50	6.1 ± 0.6 (7)*	15.3 ± 1.0 (7)*	45.2 ± 11.6 (6)*	–	–	–
100	9.3 ± 2.6 (7)*	20.3 ± 2.5 (7)*	–	–	–	–
Locomotor activity (LA) (grades)						
0 (control)	0.72 ± 0.06 (42)		0.68 ± 0.06 (42)	0.46 ± 0.07 (42)	0.93 ± 0.06 (42)	0.71 ± 0.06 (42)
6.25	0.48 ± 0.07 (42)*	0.53 ± 0.07 (42)*	0.57 ± 0.07 (42)	0.66 ± 0.07 (42)*	0.98 ± 0.17 (36)	1.69 ± 0.16 (36)*
12.5	0.69 ± 0.08 (42)	0.63 ± 0.09 (42)	0.63 ± 0.19 (42)	1.48 ± 0.15 (30)*	0.58 ± 0.09 (30)*	0.35 ± 0.08 (30)*
25	0.64 ± 0.06 (42)	0.61 ± 0.06 (42)	1.94 ± 0.13 (42)*	4.0 ± 0.00 (36)*	Immeasurable (1)	–
50	0.79 ± 0.06 (42)	0.79 ± 0.06 (42)	4.00 ± 0.00 (36)*	–	–	–
100	0.95 ± 0.07 (42)*	2.23 ± 0.11 (42)*	–	–	–	–

Number of analyzed fish (for PFA and CR) or total amount of measurements (for LA) is presented in brackets. Asterisks (*) denote values significantly different from control ($p \leq 0.05$). Dashes indicate total fish mortality

copper concentrations as low as 0.006–0.015 mg/L (threshold 0.009 mg Cu/L). Henry and Atchison (1986) found that CR was the most sensitive indicator of all the behaviors they monitored in bluegills *Lepomis macrochirus* to copper (threshold 0.034 mg Cu/L). Kazlauskienė and Vosylienė (1990) reported a significant increase of CR in rainbow trout *Oncorhynchus mykiss* within 10–30 min of being exposed to sublethal copper concentrations as low as 0.005–0.01 mg/L (threshold 0.004 mg Cu/L). Summarizing these data it should be noted that coughing threshold concentrations for copper generally fell between 0.006 and 0.09 of the 96-h LC50.

Atchison et al. (1987) concluded that although the ecological significance of cough response in fish is not clear, it could be predictive for lowest-observed-effect-concentrations usually derived from early life stage chronic toxicity tests. They stated that comparisons of behavioral responses to classical toxicity test endpoints should be made to evaluate the sensitivity of behavioral tests.

The data obtained here also confirm that CR was the most sensitive and informative parameter in roach among the responses studied. There is no doubt that its threshold value is much lower than the lowest EBM concentration tested and corresponds to the sublethal concentration range (<0.43 of 96-h LC50).

Although the coughing response in roach was found to be the most promising response, it is evident that the other behavioral responses are also sensitive indicators of heavy metal toxicity, and meet the criteria as rapid bioassay tools for early warning systems for pollution as discussed by Van der Schale et al. (2001). The detection response was found to be highly specific, but is usually disregarded by researchers as a behavioral parameter. Also, although it indicated only acutely lethal EBM concentrations, it could be very informative and a useful tool to provide rapid answers (in a few seconds) on water potential toxicity levels.

Overall, test-subjects need to be more than just easily available and maintainable. Their responses must be sensitive not only to acutely toxic pollutant levels, but also provide information about sublethal concentrations. Moreover, the objective of aquatic toxicity tests is to estimate the “safe” or “no-effect” concentration of effluents or pure compounds, which is defined as the concentration which will permit normal propagation of fish and other aquatic life in the receiving waters (US EPA 2002). Presumably, the roach CR could be a useful and rapid endpoint for predicting such concentrations, and could be successfully used in bioassay testing of treated and untreated industrial and municipal wastewaters containing heavy metals before they are discharged into receiving waters. Consequently, further more exhaustive investigation into the effects of heavy metals, as well as other priority toxicants, on the behavioral responses of roach is needed. Comparisons with the behavioral sensitivity of universally recognized test fish species also should be carried out.

Acknowledgments This work was funded by Research Council of Lithuania, Project No. MIP-58/2010-2011.

References

Alabaster JS, Lloyd R (1982) Water quality criteria for freshwater fish. FAO and Butterworths, London

ASTM (2008a) Standard guide for measurement of behavior during fish toxicity tests. E1711-95(2008). ASTM International, West Conshohocken, PA

ASTM (2008b) Standard guide for ventilatory behavioral toxicology testing of freshwater fish. E1768-95(2008). ASTM International, West Conshohocken, PA

Atchison GJ, Henry MG, Sandheinrich MB (1987) Effects of metals on fish behavior: a review. Environ Biol Fish 18:11–25

De Boeck G, Vlaeminck A, Balm PH, Lock RA, De Wachter B, Blust R (2001) Morphological and metabolic changes in common carp, *Cyprinus carpio*, during short-term copper exposure: interactions between Cu^{2+} and plasma cortisol elevation. Environ Toxicol Chem 20:374–381

Drummond RA, Spoor WA, Olson GF (1973) Some short-term indicators of sublethal effects of copper on brook trout, *Salvelinus fontinalis*. J Fish Res Board Can 30:698–701

Eisler R. (1998a) Copper hazards to fish, wildlife and invertebrates: a synoptic review. Biological science report USGS/BRD/BSRN-1997-0002, Washington, DC

Eisler R (1998b) Nickel hazards to fish, wildlife and invertebrates: a synoptic review. Biological science report USGS/BRD/BSR-1998-0001, Laurel, MD

Ellgaard EG, Ashley SE, Langford AE, Harlin DC (1995) Kinetic analysis of the swimming behavior of the goldfish *Carassius auratus* exposed to nickel: hypoactivity induced by sublethal concentrations. Bull Environ Contam Toxicol 55:929–936

Flerov BA (1989) Ecological and physiological aspects of toxicology in fresh-water animals (in Russian). Nauka, Leningrad

Gagnon A, Jumarie C, Hontela A (2006) Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout *Oncorhynchus mykiss*. Aquat Toxicol 78:59–65

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719

Henry MG, Atchison GJ (1986) Behavioral changes in social groups of bluegills exposed to copper. Trans Am Fish Soc 115:590–595

Kane AS, Salierno JD, Brewer SK (2005) Fish models in behavioral toxicology: automated techniques, updates and perspectives. In: Ostrander GK (ed) Methods in aquatic toxicology, 2nd edn. Lewis Publishers, Boca Raton, pp 559–590

Kazlauskienė N, Vosylienė MZ (1990) Effect of copper on respiratory system of rainbow trout (in Russian). In: Andrušaitis GP et al. (eds) Experimental aquatic toxicology, vol 14. Zinatne, Riga, pp 136–144

Könemann H (1981) Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. Toxicology 19:229–238

Lemly AD (1983) A simple activity quotient for detecting pollution-induced stress in fishes. Environ Tech Lett 4:173–178

Little EE, Finger SE (1990) Swimming behavior as an indicator of sublethal toxicity in fish. Environ Toxicol Chem 9:13–19

Scherer E (1992) Behavioural responses as indicators of environmental alterations: approaches, results, developments. J Appl Ichthyol 8:122–131

Scherer E, Harrison SE, Brown SB (1986) Locomotor activity and blood plasma parameters of acid-exposed lake whitefish *Coregonus clupeaformis*. Canadian J Fish Aquat Sci 43:1556–1561

SCORECARD (2005) Water quality indicators. http://www.scorecard.org

Svecevičius G (2005) Behavioral responses of rainbow trout *Oncorhynchus mykiss* to sublethal toxicity of a model mixture of heavy metals. Bull Environ Contam Toxicol 74:845–852

Svecevičius G (2010) Acute toxicity of nickel to five species of freshwater fish. Pol J Environ Stud 19:453–456

Svecevičius G, Vosylienė MZ (1996) Acute toxicity of copper to common fishes of Lithuania. Ekologija 2:17–21

Tudorache C, Jordan AD, Svendsen JC et al (2009) Pectoral fin beat frequency predicts oxygen consumption during spontaneous activity in a labriform swimming fish *Embiotoca lateralis*. Environ Biol Fish 84:121–127

US EPA (2002) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th edn. EPA-821-R-02-013. Office of Water. Washington, DC

US EPA (2006) National recommended water quality criteria. Office of Water. Office of Science and Technology (4304T). Washington, DC

US EPA (2007) Aquatic life ambient freshwater quality criteria—copper. 2007 Revision. EPA-822-R-07-001. Office of Water. Office of Science and Technology. Washington, DC

US EPA (1984) Ambient water quality criteria for copper—EPA-440/5-84-031. Office of Water Regulations and Standards, Washington, DC

USEPA (1986) Ambient water quality criteria for nickel—EPA-440/5-86-004. Office of Water Regulations and Standards, Washington, DC

Van der Schalie WH, Shedd TR, Knechtges PL, Widder MW (2001) Using higher organisms in biological early warning systems for real-time toxicity detection. Biosens Bioelectron 16:457–465

Waser W, Bausheva O, Nikinmaa M (2009) The copper-induced reduction of critical swimming speed in rainbow trout *Oncorhynchus mykiss* is not caused by changes in gill structure. Aquat Toxicol 94:77–79