To link to this article: DOI:10.1016/j.aquatox.2007.02.018
http://dx.doi.org/10.1016/j.aquatox.2007.02.018

To cite this version: Roussel, Hélène and Joachim, Sandrine and Lamothe, Sylvain and Palluel, Olivier and Gauthier, Laury and Bonzom, Jean-Marc *A long-term copper exposure on freshwater ecosystem using lotic mesocosms: Individual and population responses of three-spined sticklebacks (Gasterosteus aculeatus).* (2007) Aquatic Toxicology, vol. 82 (n°4). pp. 272-280. ISSN 0166-445X

A long-term copper exposure on freshwater ecosystem using lotic mesocosms: Individual and population responses of three-spined sticklebacks (*Gasterosteus aculeatus*)

Hélène Roussel [a,b,*], Sandrine Joachim [a], Sylvain Lamothe [a], Olivier Palluel [a], Laury Gauthier [b], Jean-Marc Bonzom [a,1]

[a] *Ecotoxicological Risk Assessment Unit, INERIS, Parc technologique ALATA, 60550 Verneuil-en-Halatte, France*

[b] *Laboratoire d'Ecologie Fonctionnelle, UMR 5245 CNRS-Université Toulouse 3-INPT, 29 rue Jeanne Marvig, 31000 Toulouse, France*

Abstract

Three-spined stickleback (*Gasterosteus aculeatus*) was used as the highest trophic level predator in an outdoor mesocosm study assessing the effect of environmentally realistic copper concentration (0, 5, 25 and 75 $\mu g\,L^{-1}$) over 18 months of continuous exposure. Condition factor, organosomatic indices (HIS, GSI and SSI) as well as copper bioaccumulation in the liver were measured at 15 days, 2, 4, 6, 10, 14 and 18 months after the beginning of the contamination. Population monitoring was realised after 6 and 18 months of contamination, allowing two reproduction periods to be measured. Results showed that condition factor was affected at medium and high copper concentrations and HSI was sporadically affected in all copper exposure, depending on the sex of the fish. GSI did not show any significant differences and SSI was lowered in the medium and high copper levels. Bioaccumulation was significantly different in males and females and fluctuated with season. A negative correlation was observed between copper bioaccumulation in the liver and fish size and a positive correlation with nominal copper concentration in the water was found. There was a negative correlation between condition factor, organosomatic indices and bioaccumulation in the liver. Population monitoring showed a significantly higher fish mean length after 6 months and a higher abundance after 18 months of exposure at the highest copper level. We conclude that indirect effects such as food and habitat availability or lower predation pressure on eggs and juveniles might have led to higher stickleback population abundances at the highest copper level. This highlights the need to study all the trophic levels when monitoring ecosystem health. Considering the population and the individual responses after 18 months of copper exposure, the NOEC for three-spined sticklebacks was 25 $\mu g\,L^{-1}$ (or 20 $\mu g\,L^{-1}$ if we consider the average effective concentration), with a LOEC of 75 $\mu g\,L^{-1}$ (or 57 $\mu g\,L^{-1}$, AEC).

Keywords: Sticklebacks; Bioaccumulation; Organosomatic index; Size frequency; Copper; Experimental channel

1. Introduction

Copper is an essential trace element required, for example, for the metabolic functioning of proteins. However, it becomes toxic when in excess (Eriksson and Weeks, 1994). Studies on various fish species have shown that copper is an osmoregulatory toxicant (Stagg and Shuttleworth, 1982) decreasing sodium influx (Grosell and Wood, 2002). Only 2 h of copper exposure at low concentrations caused a reduced branchial sodium transport affinity indicating competitive interaction between copper and sodium transport systems. The no observed effect concentrations (NOEC) for fish are between 4 and 120 $\mu g\,L^{-1}$ for 14 species (Grosell et al., 2002).

The management of water quality of streams in urban, industrial and agricultural areas requires an understanding of the ecological effects of metals on natural populations of organisms in aquatic ecosystems (Richardson and Kiffney, 2000). Ecotoxicity tests focus mainly on laboratory single-species toxicity experiments, which lack ecological realism, especially for streams because most of the test organisms are not indigenous to running waters (Norton et al., 1992; Baird and Burton, 2001). Stream mesocosms with indigenous stream organisms allow the study of a wide range of physical, chemical and

* Corresponding author. Present address: Laboratory of Soils and Environment, ISA, 48 boulevard Vauban, 59000 Lille, France. Tel.: +33 3 28 38 48 48; fax: +33 3 28 38 48 47.

E-mail address: h.roussel@isa-lille.fr (H. Roussel).

[1] Present address: Laboratory of Radioecology and Ecotoxicology, IRSN, Bât. 186, BP 3, 13115 Saint Paul Lez Durance Cedex, France.

biological properties of natural streams (Lamberti and Steinman, 1993).

To our knowledge no previous study has evaluated the impact of copper on fish in experimental stream. Few studies in experimental systems have monitored individual and population responses of a high trophic level organism such as fish (Shaw et al., 1995; Girling et al., 2000).

A long-term experiment was performed in outdoor lotic mesocosms to study copper effects on the ecosystem. The individual and population responses of a fish species were monitored over an 18-month period, in addition to studies of primary producers, invertebrates and decomposers (Roussel, 2005; Roussel et al., 2007).

A single fish species, the three-spined stickleback (*Gasterosteus aculeatus*), was present in the mesocosms. This species was chosen because of a promising biomonitoring quality (Bervoest et al., 2001; Sanchez et al., 2007) due to its wide distribution with a limited home range (Pottinger et al., 2002). This species is a small euryhaline fish of the family Gasterosteidae occupying waters of the temperate and boreal climate zones of the northern hemisphere (Wootton, 1984; Bell and Foster, 1994). Their life span show considerable inter-population variation but is for many populations not more than 2 years, with sexual maturity reached at 1 year (Wootton, 1984). They are used as an effluent-monitoring species in Canada and the United States (Environment Canada, 1990). They are good sentinel fish species, because the resident populations tend to be representative of the environment from which they have been sampled because they are relatively sedentary (Pottinger et al., 2002). In addition to this, their low economic and recreational values make them easily available.

This study investigated the effect of chronic copper exposure on three-spined stickleback individuals and population over a 2-generation period. Copper bioaccumulation in the liver, body condition factor, organosomatic indices, population abundance and length–frequency distribution were quantified to evaluate the effects of exposure to copper.

2. Materials and methods

2.1. Experimental system

This experiment was performed using 12 outdoor experimental channels (INERIS, Verneuil-en-Halatte, France), 20 m long and 1 m wide. Water depth was 30 cm, with a pebble substrate in the first 10 m and 70 cm, with fine sediments in the last 10 m. Mesocosms were set up 6 months before the experiment with artificial and natural sediments, zooplankton, phytoplankton, periphyton, controlled numbers of macrophytes, macroinvertebrates and a single species of fish (*G. aculeatus* Linnaeus), coming from a nearby unpolluted stream. Seventy young-of-the-year and adult low-plated morph sticklebacks (mean size = 35 mm, min–max = 20–57 mm, S.D. = 6.6) were introduced in each mesocosms between October and November 2001. Copper contamination started on the 15 April 2002 and lasted until the 15 October 2003. Continuous nominal concentrations of copper at 5, 25 and 75 $\mu g\,L^{-1}$ were applied in triplicates while three mesocosms served as controls. The average effective concentrations (AEC) of dissolved copper found in each treatment (control: 5, 25 and 75 $\mu g\,L^{-1}$) were, respectively, <0.5, 4 (±0.4), 20 (±0.7), and 57 (±1.1) $\mu g\,L^{-1}$ (±S.D.) following an integration method from Van Wijngaarden et al. (1996). For details on water and sediment quality see Roussel et al. (2007).

2.2. Condition factor and organosomatic indices

Three-spined sticklebacks were sampled at 15 days, 2, 4, 6, 10, 14 and 18 months after the beginning of the contamination, corresponding, respectively, to May, mid-June, mid-August, mid-October 2002, mid-February, mid-June and mid-October 2003. Fish were caught with plastic traps baited with frozen chironomid larvae. Ten fish of similar size class were randomly chosen from each mesocosms. They were sacrificed to obtain liver, gonad and spleen wet weights. The total length, weight and gender of fish were recorded as well.

Condition factor (CF) was calculated as fish weight (g)/length^3 (mm) × 10^5. Organosomatic indices such as hepatosomatic index (HSI), gonadosomatic index (GSI) and spleenosomatic index (SSI) were calculated as organ weight (mg)/fish weight (mg) × 100.

2.3. Copper bioaccumulation in liver

After weighing, the liver copper concentration was measured. Care was taken to use acid-rinsed plastic instruments to avoid copper contamination of the samples. Liver was homogenized in 200 μL of ice-cold phosphate buffer (100 mM, pH 7.8) supplemented with 20% (v/v) glycerol and 0.2 mM phenylmethylsulfonyl fluoride as a protease inhibitor. A subsample of 20 μL was taken and stored at −80 °C until analysis. Hepatic copper concentration was measured after acid digestion by an Inductively Coupled Plasma-Atomic Emission Spectrometry (Ultima, Jobin Yvon Horiba, NJ, USA), following the norm NF EN ISO 11885 (1998). Results were expressed as μg of copper per g of wet liver weight.

2.4. Population monitoring

Fish population was monitored in October 2002 and 2003 by a major fishing effort to catch most of the individuals. Thirty plastic traps were placed per mesocosm, baited with chironomid larvae and retrieved at least twice a day. The captured fish were kept aside until the end of the catch. Fishing was continued for 10 days. We estimated that nearly all the fish were caught as the traps were often empty after 7 days and no more fish were seen in the mesocosms. Total length and weight were recorded and fish were then returned to the mesocosms. Monitoring was performed in October to avoid perturbation during the breeding season (April–August).

2.5. Data analysis

Fish, liver, gonad and spleen weight, as well as copper bioaccumulation in the liver were tested for differences between sex, dates and copper treatments using a factorial ANCOVA, with fish length as covariate, (STATISTICA 6.0, Statsoft, Tulsa, OK, USA). The dependent variable (fish, organ masses and copper in the liver) and the covariate (fish length) were log transformed before analysis. When significant, a Williams test was performed per date using condition factor and organosomatic indices to detect which treatment differed from the control. This test assumes an increasing effect for an increasing dose (Williams, 1972) and is detailed in Roussel et al. (2007). It was performed using TOXSTAT (release 3.0) software. A multiple correlation analysis was performed between copper bioaccumulation in the liver and the other variables such as fish length, time after contamination, gender, liver weight, AEC of copper, season, condition factor and organosomatic indices. The duration of exposure was expressed as the number of months after the beginning of the contamination (15 April 2002) and the season was expressed as non-breeding season (September–March) and breeding season (April–August). Differences between control and treated mesocosms for mean fish length and population abundance were tested in October 2002 and 2003 using the Williams test. In addition, NOEC was calculated based on the individual and population responses. Results were considered valid if the treatment effect was observed for at least two consecutive sampling dates (Hartgers et al., 1998).

3. Results

3.1. Condition factor and organosomatic indices

The factorial ANCOVA showed that body weight and liver mass were significantly affected by sex, treatment and date and by interaction between treatment and date and between date and sex (Table 1). Gonad mass was significantly affected by sex and date and by their interaction (Table 1). Spleen weight was significantly affected by treatment and date and by their interaction (Table 1).

The Williams test per date showed significantly lower female CF for the highest copper exposure level on the last sampling date (Fig. 1). Males CF were significantly lower in the highest copper exposure level at 4, 6, 10 and 18 months after the start of the exposure and in the medium copper level at 2 and 10 months after the start of the exposure. Female HSI was significantly higher in the highest copper exposure level than in the controls 14 months after the start of the exposure (Fig. 2). Male HSI was significantly lower in all copper treatments than in controls 4 months after the start of the exposure. Female and male GSI showed no significant differences among treatments during the experiment (Fig. 3). Fish in the medium copper level had significantly lower SSI than control fish at 4 months of contamination and in the highest copper exposure level SSI was reduced both at 4 and 6 months of contamination (Fig. 4).

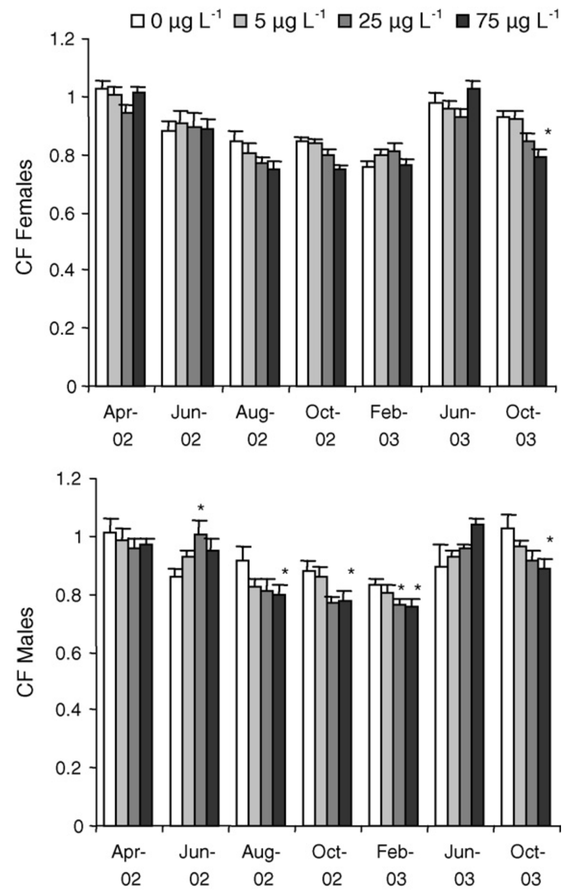

Fig. 1. Body condition factor for female and male three-spined sticklebacks chronically exposed to copper. Mean and standard error of mean are shown. Asterisks indicate significant difference (Williams test, $P<0.05$).

Table 1
Results of the ANCOVA for the significant differences of three-spined sticklebacks body mass, organ weights and copper bioaccumulation in the liver, depending on sex, treatment and date, with body length as the covariate

	Fish weight			Liver weight		Gonad weight		Spleen weight		Copper bioaccumulation in liver	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Sex	1	6.97	0.008	126.53	0.000	1779.79	0.000	3.39	0.066	154.54	0.000
Treatment	3	4.71	0.000	3.68	0.012	0.13	0.940	2.86	0.036	401.89	0.000
Date	6	44.86	0.002	50.71	0.000	48.19	0.000	4.25	0.000	345.37	0.000
Treatment × date	18	3.45	0.000	4.83	0.000	1.17	0.281	2.04	0.006	8.06	0.000
Treatment × sex	3	0.33	0.806	1.04	0.372	2.60	0.051	0.22	0.881	3.04	0.028
Sex × date	6	2.67	0.014	20.57	0.000	102.46	0.000	0.28	0.946	28.58	0.000
Treatment × date	18	0.96	0.510	0.84	0.648	1.18	0.272	0.75	0.761	1.78	0.024

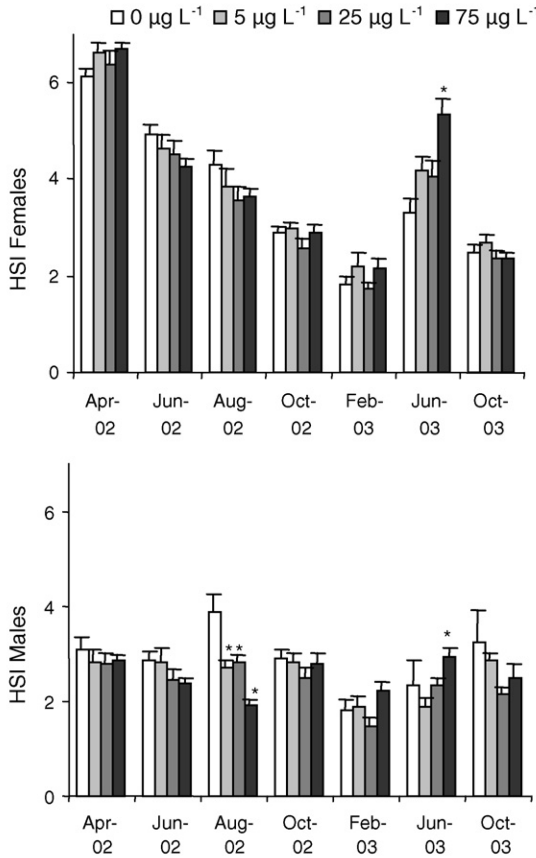

Fig. 2. Hepatosomatic index for female and male three-spined sticklebacks chronically exposed to copper. Mean and standard error of mean are shown. Asterisks indicate significant difference (Williams test, $P < 0.05$).

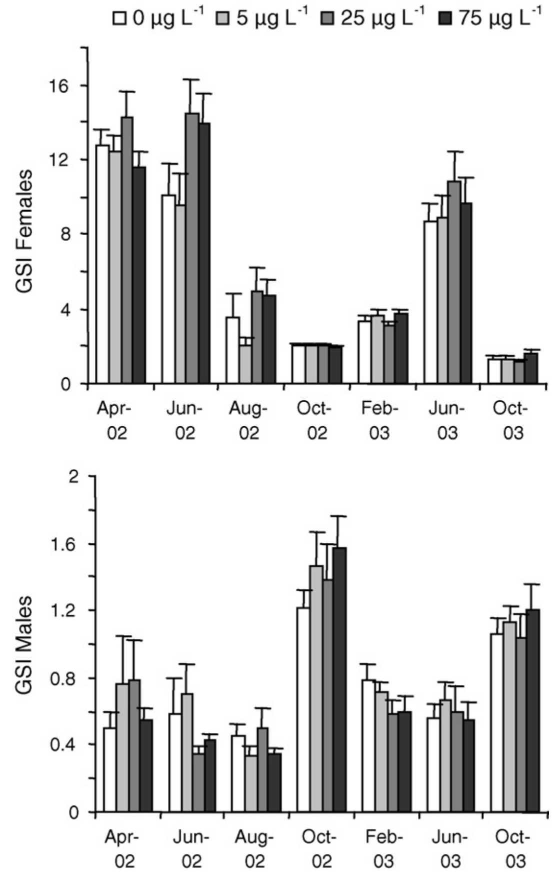

Fig. 3. Gonadosomatic index for female and male three-spined sticklebacks chronically exposed to copper. Mean and standard error of mean are shown. Asterisks indicate significant difference (Williams test, $P < 0.05$).

3.2. *Copper bioaccumulation in the liver*

The factorial ANCOVA showed that copper bioaccumulation in the fish liver was significantly affected by sex, treatment and date and by interaction between treatment and date, between date and sex and between treatment, date and sex (Table 1). Significant correlations were obtained between the copper bioaccumulation in the liver and the time of exposure, the fish length, the liver weight, the AEC, the season, the condition factor and the organosomatic indices (Table 2). Although it is difficult to separate out the effects of season and duration of exposure, there was a significant negative correlation between fish size and copper bioaccumulation for all control and treated groups. In June 2002, control fish of 56.1 mean length (mm) had a basal level of 74.5 µg of copper per g of wet liver while in June 2003, control fish of 43.3 mean length (mm) had a basal level of 336.1 $\mu g\ g^{-1}$ (Fig. 5). In addition to the size effect, copper bioaccumulation in the control and treated fish liver showed seasonal variation. A

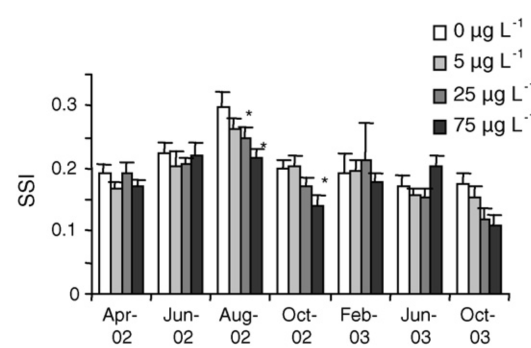

Fig. 4. Spleenosomatic index for three-spined sticklebacks chronically exposed to copper. Mean and standard error of mean are shown. Asterisks indicate significant difference (Williams test, $P < 0.05$).

Table 2
Correlation coefficients and P values for each parameters related to copper bioaccumulation in the liver

	Season	Fish length	HSI	Liver weight	AEC	BCF	Time post exposure	GSI	SSI	Gender
r	−0.45	−0.43	−0.41	−0.39	0.33	−0.31	0.26	−0.20	−0.09	0.00
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.984

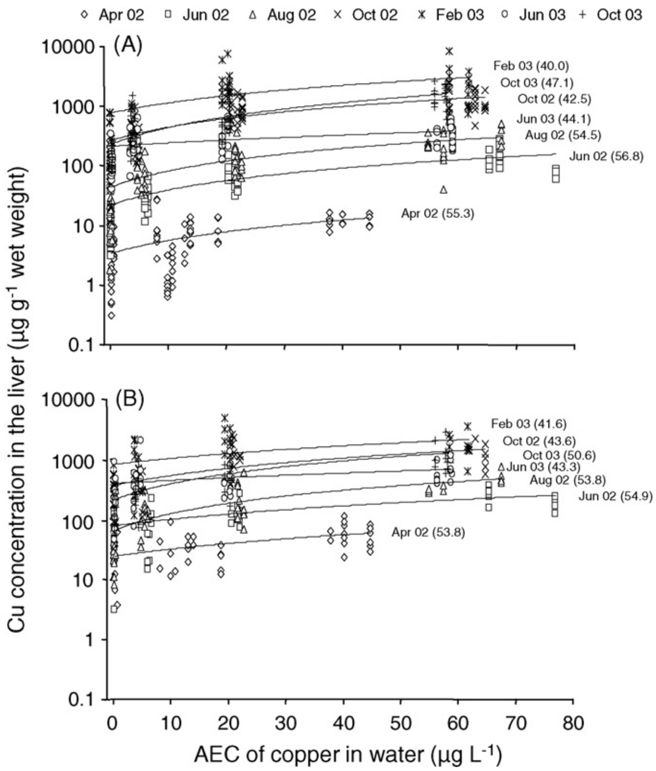

Fig. 5. Copper concentration in liver of female and male three-spined sticklebacks chronically exposed to copper in µg of copper per g of wet liver weight per dates. Number next to the date indicates the fish mean length (mm).

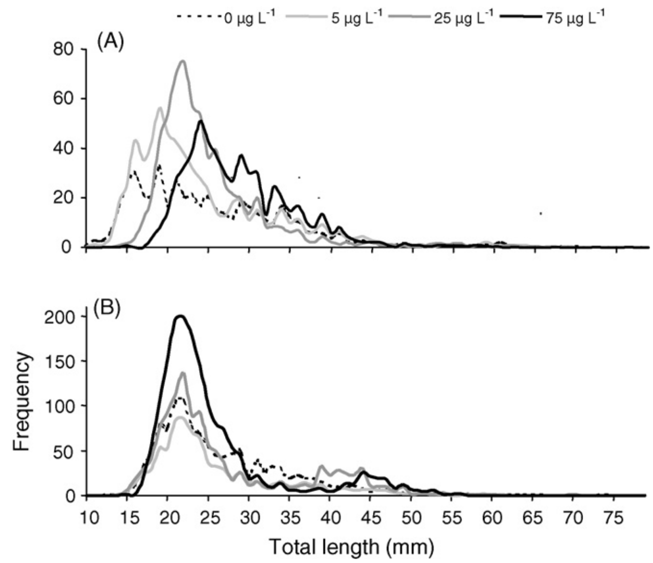

Fig. 6. Size-class frequencies of the three-spined sticklebacks population in (A) October 2002 and (B) October 2003 per treatment (0, 5, 25 and 75 $\mu g\,L^{-1}$).

higher copper content was measured during non-breeding season (autumn and winter) in both control and treated groups.

3.3. Population monitoring

From the 70 sticklebacks introduced in each mesocosm before the beginning of the contamination we obtained, 1 and 2 years later, about 500 and 1000 fish per mesocosm, respectively. Length–frequency distributions were obtained from the two population samples taken in October 2002 and 2003, respectively, after 6 and 18 months of contamination. Young-of-the-year were estimated to be below 35 mm of length (Wootton, 1984). For the October 2002 sample, there was a shift in the mode for the young-of-the-year towards a longer length class in the medium and high copper levels (Fig. 6). The percentage of size frequency in the class 10–19 mm was 33 and 2% for the control and the high copper level, respectively. On the opposite, there was 37, 70 and 59% of fish in the class of size 20–29 mm for the control, medium and high copper level, respectively. In October 2003, there was no shift in the position of the mode, but the frequency in the modal size class of young-of-the-year was higher in the high copper level than in controls. For the controls and the highest copper concentration, there were 56 and 72% of fish in the class of size 20–29 mm, respectively.

In October 2002 there was no significant difference in total abundance of fish between control and treated mesocosms. However, the mean length of fish was significantly higher in the highest copper exposure level than in the control (Table 3). In October 2003, the total abundance of fish was significantly higher in the highest copper exposure level than in the control, while there was no difference in the mean length of fish between control and treated mesocosms (Table 3).

4. Discussion

The results show that stickleback condition factor and organosomatic indices were slightly affected by copper and that they are negatively correlated with bioaccumulation in the liver. However, despite of the elevated level of copper bioaccumulated in the fish liver the population monitoring indicates that stickle-

Table 3
Population monitoring realised in October 2002 and in October 2003

Treatment ($\mu g\,L^{-1}$)	2002					2003				
	Abundance		Length (mm)			Abundance		Length (mm)		
	Mean	±S.D.	Mean	±S.D.	Min–max	Mean	±S.D.	Mean	±S.D.	Min–max
0	638	242	26.4	9.3	11–71	1185	65	27.9	8.5	12–80
5	574	123	24.7	8.6	8–68	823	63	27.2	8.4	15–75
25	483	65	26	6.8	15–66	1208	407	28.2	9.3	15–73
75	480	29	29.7*	6.7	16–61	1685*	224	26.7	7.9	16–60

Mean, minimum, maximum and standard deviation of abundance and length are given. Asterisks indicate significant difference (Williams test, $P < 0.05$).

back had a longer length after 6 months and a higher abundance after 18 months in the highest copper exposure level suggesting possible indirect effects.

4.1. *Condition factor and organosomatic indices*

Condition factor is an indicator of the overall fish condition and it reflects fish shape and energy reserves. It is often used to evaluate fish stress (Lohner et al., 2001; Eastwood and Couture, 2002). It has been stated previously that the condition factor can fluctuate with physiological development, sexual maturation, season and geographic location (Goede and Barton, 1990). In our experiment CF was depressed for females and males on several dates in the medium and the highest copper exposure level. A decline in condition factors is usually interpreted as a depletion of energy resources, such as stored glycogen or body fat (Goede and Barton, 1990). Eastwood and Couture (2002) reported that a decreased condition factor of metal-contaminated fish could be explained either by direct effects on juveniles (metabolic impacts) or by an impoverishment of the food chain, both events leading to decreases in fish growth and overall condition. They observed that yellow perch from metal-contaminated sites had lower values for indicators of physical condition than fish from cleaner lakes (Eastwood and Couture, 2002). In another study, condition factors have been shown to decrease in coho salmon when chronically exposed to 140 $\mu g\,L^{-1}$ of copper in water with 280 $mg\,L^{-1}$ hardness and with a pH around 7.7 (Buckley et al., 1982). In our experiment, a lower impact of copper on the condition factor in females than in males was observed. Perkins et al. (1997) also observed a decreasing tendency of the male channel catfish condition factor while no change was seen in females under copper exposure.

HSI was depressed in all treated mesocosms in males after 4 months of exposure and it was increased in the highest copper exposure level in both males and females after 14 months of exposure. Although Friedmann et al. (2002) reported a significant decrease in HSI in fish from mercury-contaminated sites, many authors have not found significant changes in HSI under copper exposure (Perkins et al., 1997; Eastwood and Couture, 2002; Sanchez et al., 2005). Kamunde and Wood (2003) showed that HSI in juvenile rainbow trout was not affected by dietary copper exposure at 15 $\mu g\,g^{-1}$ of fish per day. However, in their experiment, HSI was affected by fish ration, being low in the fish on lower ration and higher in the fish on high ration. Ali and Wootton (1999) state that there is evidence that the stickleback liver acts as a storage organ. Stickleback liver shows changes in size that are correlated both with the nutritional state of the fish and with the reproductive cycle (Wootton, 1984). HSI is correlated with the general nutritional status and the observed decrease in HSI could also be the result of limited food availability (Friedmann et al., 2002). Thus, variation of HSI measured in our experiment could be related more to food availability than to direct copper effect. This is confirmed by the lower abundance of the invertebrates which are stickleback prey, in the highest copper exposure level in August 2002 and the higher abundance in June 2003 (Roussel, 2005). In addition, a seasonal variation was observed in females with a high HSI in spring 2003, at the beginning of the breeding period. Banks et al. (1999) reported significant seasonal variations in channel catfish HSI with elevated levels from October to April and then decline to their lowest levels 2 months prior to spawning. It is characteristic of the period of exogenous vitellogenesis (Banks et al., 1999). Therefore, in our experiment, higher HSI of copper-exposed females, reported in June 2003, could result from a delayed reproduction period compared to controls or to higher food availability, allowing more energy storage in the liver. As the males showed the same significant difference as females, it was probably the food availability that was the origin of a higher HSI.

GSI showed no significant differences between treatments in either the females or the males but showed significant seasonal variation. Banks et al. (1999) reported significant seasonal variation of channel catfish GSI levels with an increase from March to June, coinciding with the acceleration of vitellogenesis in the liver and incorporation of VTG into the growing oocyte. In our experiment, high GSI in females corresponded to the spawning period, which is quite extended because three-spined sticklebacks have the property to spawn several times during a breeding season (Wootton, 1976).

SSI did not show gender differences and was significantly lower in the highest copper exposure level than in the controls in August and October 2002 which suggests a lower activity of the immune system. The response of the immune system is often used to assess the toxic effects of chemicals (Weeks et al., 1992).

4.2. *Copper bioaccumulation in liver*

A positive correlation between the average effective concentrations of copper in the water and copper concentrations in the liver was measured. Bervoest et al. (2001) showed that the liver of three-spined stickleback was the tissue with the highest copper content. They reported a positive correlation between copper in the liver and copper in the invertebrates but not between water or sediment and liver copper. The reported measured copper concentration in the liver was up to 50 $\mu g\,g^{-1}$ of dry weight (corresponding to approximately 18 $\mu g\,g^{-1}$ wet liver) for water concentrations between 0.6 and 7.4 $\mu g\,L^{-1}$, sediment concentration from 0.3 to 10.2 $\mu g\,g^{-1}$ and invertebrate concentrations from 19.9 to 63.2 $\mu g\,g^{-1}$ dry weight. Note that the copper level in water and sediment was low compared to our study. Sanchez et al. (2005) reported the copper concentration in three-spined stickleback liver to be around 45 $\mu g\,g^{-1}$ liver dry weight after 21 days of 100 $\mu g\,L^{-1}$ copper exposure in a similar water quality as ours. These values are extremely low compared with our findings. However, in our experiment, fish were simultaneously exposed to water, sediment and food. Moreover, the time of exposure was much longer, allowing extended bioaccumulation to occur.

Bioaccumulation was correlated with season, fish size, liver weight, condition factor, organosomatic indices and time of exposure. The understanding of such variations is complex as copper is an essential mineral with cellular and tissue levels subject to metabolic regulation (McGeer et al., 2000). As an example of homoeostatic regulation of copper, the metallothioneins have a high inducibility by metal exposure and to be especially valuable in the detoxification and transport of heavy metals (Hammer, 1986). Seasons have been shown to affect metallothionein response to metal exposure (Rotchell et al., 2001). As metallothionein acts in the cellular regulation of copper this could explain, in part, the lower copper content observed in spring and summer. Moreover, Banks et al. (1999) reported that there were significant seasonal variations in total hepatic zinc concentrations with a significant elevation immediately after spawning. Seasonal variation of liver copper was suspected to be related to the breeding period as the spring and summer seasons differed from the non-breeding period, autumn and winter. Females had a lower liver copper content during the breeding season (spring and summer) than males. No differences were observed in autumn and winter, during the non-breeding seasons. It has previously been shown that levels of radioactive cesium (^{137}Cs) in whole-body of largemouth bass differed between sexes, with a lower content in females than in males (Peles et al., 2000). Lower copper content in the liver could be related to a female-specific detoxification mechanism that is associated with reproduction. A possible mechanism involves the distribution of extra-hepatic copper in females to a less critical site (Perkins et al., 1997). Vitellogenin, a yolk precursor protein produced in the liver, is involved in metal binding (Zn and Cu) in winter flounder (Fletcher and Fletcher, 1980). A fraction of the copper could have been bound by vitellogenin in the liver and subsequently transported and stored in the ovaries (Perkins et al., 1997), explaining a decrease in female copper concentration in the liver during breeding period.

A negative correlation between fish size and the copper concentration in the liver was observed. This effect was difficult to separate from season and the time of exposure. Fish in June 2003 were smaller and had a higher copper concentration than fish in June 2002 that were larger and had a lower copper concentration. However, the time of exposure was different (2 and 14 months, respectively) and the fish generation was not the same. Fish sampled after 14 months of exposure were 1-year fish, which were born at the beginning of the contamination and that grew up under copper exposure. Thus it was difficult to identify a clear size effect, even if it has previously been shown that copper concentrations in the liver were correlated with fish size (Canli and Atli, 2003; Farkas et al., 2003).

4.3. *Population monitoring*

The increase in the stickleback population showed that the mesocosm ecosystem was favourable to reproduction and growth of this species. Surprisingly, the highest copper exposure level was related to a higher mean length in 2002 and a higher fish number in 2003 than the values in control mesocosm. The fish did not seem to be affected by the direct toxicity of copper. Lethal concentration for three-spined sticklebacks (LC50, 96 h) was determined to be as high as $1.49\,\mathrm{mg\,L^{-1}}$ of copper (Svecevicius and Vosyliene, 1996). This was much higher than our exposure concentrations (up to $75\,\mu\mathrm{g\,L^{-1}}$). However, a study performed in indoor aquaria, with fish taken from the control mesocosms and with similar water quality as ours revealed a mortality rate of 17% at $200\,\mu\mathrm{g\,L^{-1}}$ of copper (Sanchez et al., 2005). They stated that the surviving fish appeared stressed, with a lower mobility and a loss of appetite than observed for non-exposed fish. It showed the sensitivity of our fish population to copper at $200\,\mu\mathrm{g\,L^{-1}}$. The copper bioavailability was certainly higher in this study than in ours due to the absence of organic matter coming from plants, which binds the free copper and thus reduces its toxicity (Stiff, 1971). Similarly, an aquarium study performed by Gravenmier et al. (2005) showed a 96 h LC50 of $227.2\,\mu\mathrm{g\,L^{-1}}$ for copper for adult three-spined stickleback in water more than three times less alkaline than ours.

The higher mean length and higher abundance of fish in the highest copper exposure level than in the control mesocosm was probably related to habitat quality, providing more food, more breeding sites, less predation or less competition. Three-spined stickleback is a slow growing fish which reaches a weight of about 0.5 g and a length of about 35 mm at the end of the first year of life (Wootton, 1984). To explore if food availability was a possible factor explaining why the fish living in the more polluted mesocosms had a higher length or a higher abundance, the stomach content of 40 sticklebacks from various mesocosms was investigated after the end of the experiment (data not shown). In spring 2004, fish below 45 mm were mainly feeding on cladocerans and copepods and larger fish >45 mm were mainly feeding on gastropods, copepods and sometimes fish eggs. In autumn 2004, fish below 45 mm were essentially feeding on diatoms, fish between 45 and 60 m were also feeding on diatoms, ostracods and copepods and larger fish >60 mm were feeding mainly on gastropods. These results show both the seasonal variation and the body size effect on the fish diet. Small fish ate more planktonic prey while larger fish ate mainly benthic organisms. A segregation into pelagic and benthic feeders was previously reported (Gill and Hart, 1996). Jakobsen et al. (2003) showed that stickleback at high density can exert strong top-down control on zooplankton community structure. The predation pressure on zooplankton remains continuously high because sticklebacks may produce more than one cohort per year, because females are batch spawners (Sondergaard et al., 2000). In our experiment, cladoceran abundance decreased in spring 2002, in all control and treated mesocosms, probably due to a high predation by recently hatched fish (Roussel, 2005). An increase in macroinvertebrate abundance was observed in spring 2003 in the highest copper exposure level due to an increase of chironomidae and oligochaetes. Adult fish may have specialised in chironomid consumption and thus experienced a better breeding season due to a higher food level than in the previous year. Sticklebacks have been shown to shift from an opportunistic feeding strategy in winter to a specialist one in spring (Sanchez-Gonzales et al., 2001). Wootton (1984) reported that, as the abundance or availability of prey species changes during the year, the diet of the sticklebacks changes. It was surprising to have such a high diatoms content in the diet. Mesocosms were very rich in periphyton, growing on the plants and on the mesocosm wall (Roussel et al., 2007). This would have favoured consumption of diatoms by sticklebacks during periods with low invertebrate availability. The periphyton biomass was very high in the medium and high treatments due to the lack of gastropods grazers (Roussel, 2005). This high biomass might have been favourable to the fish, supplying plenty of food for small size class individuals like YOY and allowing a better survival.

Moreover, invertebrate predation was probably an important factor affecting the population dynamics of the sticklebacks. Leeches and gastropods eat stickleback eggs and juveniles fish are the prey of large insects such as the nymph of dragonflies, notonect and dytiscus larvae (Wootton, 1984). In comparison with the controls, leeches (erpobdellidae, glossiphoniidae), notonectidae, naucoridae and gastropods (lymaeidae, physidae) were depleted in the highest copper exposure level due to copper toxicity (Roussel, 2005). For example, during spring 2003, leeches abundance was 10 times higher in the controls than in the high treatment. The predation pressure was thus lowered in the highest copper exposure level. A few "white-spot" parasites (ciliate Ichthyophthirius) were observed at the beginning of the experiment on introduced fish, but after the first breeding season, no more parasitized fish were observed in the mesocosms. Thus, parasitism was probably not a major factor that influenced fish population dynamics.

5. Conclusion

In consideration of the population and the individual responses after 18 months of copper exposure, the no observed effect concentration value (NOEC) for the three-spined stickleback was $25\,\mu g\,L^{-1}$ (or $20\,\mu g\,L^{-1}$ if we consider the average effective concentration), with a LOEC of $75\,\mu g\,L^{-1}$ (or $57\,\mu g\,L^{-1}$ AEC). Although fish exposed to the highest copper exposure level had a high copper concentration in the liver the population abundance was not affected. It was even enhanced in the highest copper exposure level, showing the opportunistic ability of this fish, which is known to be able to adapt under various conditions. No clear pattern in the fish condition indices or in the copper bioaccumulation could explain the increase in fish abundance in the highest copper exposure level. Indirect effects like a reduction in predation pressure were suspected to have been a factor in the response of the stickleback population. Positive effects observed on stickleback population in the highest copper exposure level were probably an indirect effect of copper and not a direct positive effect. It shows the importance of monitoring all the trophic levels when assessing a toxicant effect. If we had monitored only the fish population in this study, we could have deduced that the ecosystem functioning was enhanced by copper. From the other parts of this study on primary producers, invertebrates and decomposers (Roussel, 2005; Roussel et al., 2007) we can see that it is not the case. The study revealed the complexity of indirect effects and the importance of evaluating the whole system when assessing the toxicity of pollutants.

References

Ali, M., Wootton, R.J., 1999. Effect of variable food levels on reproductive performances of breeding female three-spined sticklebacks. J. Fish Biol. 55, 1040–1056.

Baird, D.J., Burton, A.G.J., 2001. Ecological Variability: Separating Natural from Anthropogenic Causes of Ecosystem Impairment. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL, USA.

Banks, S.D., Thomas, P., Baer, K.N., 1999. Seasonal variations in hepatic and ovarian zinc concentrations during the annual reproductive cycle in female channel catfish (*Ictalurus punctatus*). Comp. Biochem. Physiol. C 124, 65–72.

Bell, M.A., Foster, S.A., 1994. The Evolutionary Biology of the Three-spine Stickleback. Oxford University Press, Oxford, UK.

Bervoest, L., Blust, R., Verheyen, R., 2001. Accumulation of metals in the tissues of three spined stickleback (*Gasterosteus aculeatus*) from natural fresh waters. Ecotoxicol. Environ. Saf. 48, 117–127.

Buckley, J.T., Roch, M., McCarter, J.A., Rendell, C.A., Matheson, A.T., 1982. Chronic exposure of coho salmon to sublethal concentrations of copper. I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol. C 72, 15–19.

Canli, M., Atli, G., 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut. 121, 129–136.

Eastwood, S., Couture, P., 2002. Seasonal variations in condition and liver metal concentrations of yellow perch (*Perca flavescens*) from a metal-contaminated environment. Aquat. Toxicol. 58, 43–56.

Environment Canada, 1990. Biological Test Method: Acute Lethality Test Using Threespine Stickleback *Gasterosteus aculeatus*. EPS 1/RM/10, Ottawa, Ontario.

Eriksson, S., Weeks, J., 1994. Effects of copper and hypoxia on two populations of the benthic amphipod *Corophium volutator* (Pallas). Aquat. Toxicol. 29, 73–81.

Farkas, A., Salanki, J., Specziar, A., 2003. Age and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Res. 37, 959–964.

Fletcher, P.E., Fletcher, G.L., 1980. Zinc- and copper-binding proteins in the plasma of winter flounders (*Pseudopleuronectes americanus*). Can. J. Zool. 58, 609–613.

Friedmann, A.S., Costain, E.K., MacLatchy, D.L., Stansley, W., Washuta, E.J., 2002. Effects of mercury on general and reproductive health of largemouth bass (*Micropterus salmoides*) from three lakes in New Jersey. Ecotoxicol. Environ. Saf. 52, 117–122.

Gill, A.B., Hart, P.J., 1996. How feeding performance and energy intake change with a small increase in the body size of the three spined stickleback. J. Fish Biol. 48, 878–890.

Girling, A.E., Tattersfield, L.J., Mitchell, G.C., Pearson, N., Woodbridge, A.P., Bennett, D., 2000. Development of methods to assess the effects of xenobiotics in outdoor artificial streams. Ecotoxicol. Environ. Saf. 45, 1–26.

Goede, R.W., Barton, B.A., 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Am. Fish Soc. Symp. 8, 93–108.

Gravenmier, J.J., Johnston, D.W., Santore, R.C., Arnold, W.R., 2005. Acute toxicity of copper to the threespine stickleback *Gasterosteus aculeatus*. Environ. Toxicol. 20, 150–159.

Grosell, M., Nielsen, C., Bianchini, A., 2002. Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. Comp. Biochem. Physiol. C 133, 287–303.

Grosell, M., Wood, C.M., 2002. Copper uptake across rainbow trout gills: mechanisms of apical entry. J. Exp. Biol. 205, 1179–1188.

Hammer, D., 1986. Metallothionein. Annu. Rev. Biochem. 55, 913–951.

Hartgers, E.M., Aalderink, G.H.R., Van den Brink, P.J., Ronald, G., Weigman, J.W., Brock, T.C.M., 1998. Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms. Aquat. Ecol. 32, 135–152.

Jakobsen, T.S., Hansen, P.B., Jeppesen, E., Gronkjaer, P., Sondergaard, M., 2003. Impact of three-spined stickleback *Gasteroseus aculeatus* on zooplankton and chl *a* in shallow, eutrophic, brackish lakes. Mar. Ecol. Prog. Ser. 262, 277–284.

Kamunde, C., Wood, C.M., 2003. The influence of ration size on copper homeostasis during sublethal dietary copper exposure in juvenile rainbow trout *Oncorhynchus mykiss*. Aquat. Toxicol. 62, 235–254.

Lamberti, G., Steinman, A., 1993. Research in artificial streams: applications, uses and abuses. J. N. Am. Benthol. Soc. 12, 313–384.

Lohner, T.W., Reash, R.J., Willet, V.E., Rose, L.A., 2001. Assessment of tolerant sunfish populations (*Lepomis* sp) inhabiting selenium-laden coal ash effluents. Ecotoxicol. Environ. Saf. 50, 203–216.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., Wood, C.M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in Rainbow Trout 1: iono-regulatory disturbance and metabolic costs. Aquat. Toxicol. 50, 231–243.

NF EN ISO 11885, 1998. Water Quality-Determination of 33 Elements by Inductively Coupled Plasma Atomic Emission Spectroscopy. AFNOR, Paris.

Norton, S.B., Rodier, D.J., Gentile, J.H., Van der Shalie, W.H., Wood, W.P., Slimak, M.W., 1992. A framework for ecological risk assessment at the EPA. Environ. Toxicol. Chem. 11, 1663–1672.

Peles, J.D., Philippi, T., Smith, M.H., Brisbin Jr., L., Gibbons, J.W., 2000. Seasonal variation in radiocesium levels of largemouth bass (*Micropterus salmoides*): implications for humans and sensitive wildlife species. Environ. Toxicol. Chem. 19, 1830–1836.

Perkins, E.J., Griffin, B., Hobbs, M., Gollon, J., Wolford, L., Schlenk, D., 1997. Sexual differences in mortality and sublethal stress in channel catfish following a 10 week exposure to copper sulfate. Aquat. Toxicol. 37, 327–339.

Pottinger, T., Carrick, T., Yeomans, W., 2002. The three-spined stickleback as an environmental sentinel: effects of stressor on whole-body physiological indices. J. Fish Biol. 61, 207–229.

Richardson, J.S., Kiffney, P.M., 2000. Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada, stream to metals in experimental mesocosms. Environ. Toxicol. Chem. 19, 736–743.

Rotchell, J.M., Clarke, K.R., Newton, L.C., Bird, D.J., 2001. Hepatic metallothionein as a biomarker for metal contamination: age effects and seasonal variation in European flounders (*Pleuronectes flesus*) from the Severn Estuary and Bristol Channel. Mar. Environ. Res. 52, 151–171.

Roussel, H., 2005. The effects of copper on structure and function of freshwater ecosystems: a lotic mesocosm study. Ph.D. Thesis. University of Toulouse, p. 249.

Roussel, H., Ten-Hage, L., Joachim, S., Le Cohu, R., Gauthier, L., Bonzom, J.-M., 2007. A long-term copper exposure on a freshwater ecosystem using lotic mesocosms: primary producer community responses. Aquat. Toxicol. 81, 168–182.

Sanchez-Gonzales, S., Ruiz-Campos, G., Contreras-Balderas, S., 2001. Feeding ecology and habitat of the three-spined stickleback, *Gasterosteus aculeatus microcephalus*, in a remnant population of northwestern Baja California, Mexico. Ecol. Freshwater Fish 10, 191–197.

Sanchez, W., Aït-Aïssa, S., Palluel, O., Ditche, J.-M., Porcher, J.-M., 2007. Preliminary investigation of multi-biomarker responses in three-spined stickleback (*Gasterosteus aculeatus* L.) sampled in contaminated streams. Ecotoxicology 16, 279–287.

Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, J.-M., Aït-Aïssa, S., 2005. Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. Environ. Toxicol. Pharmacol. 19, 177–183.

Shaw, J.L., Maund, S.J., Marshall, S.J., Hill, I.R., 1995. Fathead minnow reproduction in outdoor microcosms: an assessment of the ecological effects of fish density. Environ. Toxicol. Chem. 14, 1763–1772.

Sondergaard, M., Jeppesen, E., Aaser, H.F., 2000. Neomysis integer in a shallow hypertrophic brackish lake: distribution and predation by three-spined stickleback (*Gasterosteus aculeatus*). Hydrobiologia 428, 151–159.

Stagg, R.M., Shuttleworth, T.J., 1982. The accumulation of copper in *Platichthys flesus* L. and its effect on plasma electrolyte concentrations. J. Fish Biol. 20, 491–500.

Stiff, M.J., 1971. The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. Water Res. 5, 585–599.

Svecevicius, G., Vosyliene, M.Z., 1996. Acute toxicity of copper to common freshwater fishes of Lithuania. Ekologija 2, 17–21.

Van Wijngaarden, R.P.A., Van den Brink, P.J., Oude Voshaar, J.H., Leeuwangh, P., 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches. I. Comparison of short-term toxicity between laboratory and field. Environ. Toxicol. Chem. 15, 1133–1142.

Weeks, B.A., Anderson, D.P., Dufour, A.P., Fairbrother, A., Goven, A.J., Lahvis, G.P., Peters, G., 1992. Immunological biomarkers to assess environmental stress. In: Hugetts, R.J., Kimerle, R.A., Mehrle, J.P.M., Bergman, H.L.s. (Eds.), Biomarkers. Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publisher, Boca Raton, pp. 211–234.

Williams, D.A., 1972. The comparison of several dose levels with a zero dose control. Biometrics 28, 510–531.

Wootton, R.J., 1976. The Biology of the Sticklebacks. Academic press, London.

Wootton, R.J., 1984. The Functional Biology of Sticklebacks. Croom Helm, London.