Acute toxicity of ammonia and nitrite to different ages of Pacific cod (Gadus macrocephalus) larvae

Wei Wang*, Hua Wang*, Chunyan Yu* and Zhiqiang Jiang*

*School of Fisheries and Life Science, Dalian Ocean University, Dalian, China; †State Oceanic Administration, National Marine Environmental Monitoring Center, Dalian, China

ABSTRACT
This study was conducted to evaluate the acute toxicity of ammonia and nitrite to three developmental stages of Pacific cod (Gadus macrocephalus) larvae (11, 22, and 35 days after hatching, with mean total lengths of 4.63 ± 0.14, 5.83 ± 0.17, and 7.46 ± 0.23 mm, respectively). The results showed for the first time that the acute toxicity of ammonia and nitrite is closely related to the age of Pacific cod larvae, and the acute toxicity of ammonia or nitrite increased with increased Pacific cod larval growth. Lethal concentrations (LC50) of un-ionized ammonia nitrogen (UIAN) for a 48-h exposure in 11-day post-hatch, 22-day post-hatch, and 35-day post-hatch Pacific cod larvae were 1.72, 0.69, and 0.32 mg L−1, respectively. The 48-h LC50 of nitrite nitrogen to Pacific cod larvae 11-day post-hatch, 22-day post-hatch, and 35-day post-hatch were 831.76, 269.15, and 223.87 mg L−1, respectively. The present findings demonstrate that the acute toxicity of ammonia for Pacific cod larvae is much higher than that of nitrite.

© 2015 the author(s). Published by Taylor & Francis. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Ministry of Agriculture, China. Larvae of the same age from multiple parents were acclimated to experimental conditions in a 1200-L tank and managed according to a standardized culture protocol. Prior to each toxicity test, 20 larvae were randomly selected, and their lengths and weights were measured. The wet body weight and total length of Pacific cod larvae selected for acute toxicity tests are shown in Table 1.

The seawater used for this study was obtained from the Dalian coast of the Yellow Sea and pre-treated with a water treatment system that combined a sand filter, a UV disinfector, and an electrical heater. The water quality parameters of seawater used in all experiments are shown in Table 2. Temperature, salinity, and dissolved oxygen were determined using a YSI dissolved oxygen meter (Professional Plus Model 550A; Yellow Springs, USA), and the pH of the seawater was monitored with a pH meter (Model PB-10; Sartorius, Germany). The chemical oxygen demand, total ammonia nitrogen (TAN), and nitrite nitrogen (\(\text{NO}_2^-\)) were determined using methods based on the specification for marine monitoring – Part 4: Seawater Analysis (GB17378.4–2007). The un-ionized ammonia nitrogen (UIAN) levels in the experimental aquarium were calculated according to the concentration of TAN, water temperature, salinity, and pH values using equations from Sea Water Quality Standard (GB3097–1997).

### Table 1. Different ages of Pacific cod larvae (n = 20) for acute toxicity tests.

| Larval stage           | Mean length (mm) | Mean weight (mg) |
|------------------------|------------------|------------------|
| 11-day post-hatch      | 4.63 ± 0.14      | 17.9 ± 0.2       |
| 22-day post-hatch      | 5.83 ± 0.17      | 33.7 ± 0.5       |
| 35-day post-hatch      | 7.46 ± 0.23      | 53.9 ± 1.1       |

### Table 2. The water quality parameters of seawater used in this study.

| Parameter                     | Values            |
|-------------------------------|-------------------|
| Water temperature (°C)        | 10.0 ± 0.5        |
| Salinity                      | 29.8 ± 0.5        |
| pH                            | 8.05 ± 0.05       |
| Dissolved oxygen (mg L\(^{-1}\)) | 7.97 ± 0.21       |
| Chemical oxygen demand (mg L\(^{-1}\)) | 0.66 ± 0.25       |
| Total ammonia nitrogen (mg N L\(^{-1}\)) | 0.017 ± 0.004     |
| Nitrite (mg N L\(^{-1}\))    | 0.005 ± 0.003     |

### Table 3. Concentrations of UIAN and \(\text{NO}_2^-\) for the acute toxicity tests.

|                  | 11-day post-hatch | 22-day post-hatch | 35-day post-hatch |
|------------------|-------------------|-------------------|-------------------|
| UIAN (mg L\(^{-1}\)) | \(\text{NO}_2^-\) (mg L\(^{-1}\)) | UIAN (mg L\(^{-1}\)) | \(\text{NO}_2^-\) (mg L\(^{-1}\)) |
| 1.12             | 0.28              | 0.06              | 0.11              |
| 2.23             | 0.56              | 0.22              | 0.28              |
| 3.35             | 0.83              | 0.45              | 0.39              |
| 4.81             | 0.94              | 0.55              | 0.55              |
| 5.59             | 0.10              | 0.56              | 0.56              |
| 6.70             | 1.12              | 0.56              | 0.56              |
| 7.82             | 1000.00           | 1000.00           | 1000.00           |

Acute toxicity testing

Based on preliminary experiments to define the ranges of the toxicants, the Pacific cod larvae were acutely exposed to either ammonia or nitrite for the formal experiments. The experiments were run separately with different groups of larvae from the same acclimation tank, and both experiments included controls to which no toxicant was added. The experimental concentrations of the test solutions and the controls were all tested in triplicate. The desired test concentrations were obtained using stock solutions made with analytical grade ammonium chloride and sodium nitrite (Guoyao Chemical Reagent Company, China). Seven concentrations each of ammonia and nitrite were used for the various acute toxicity tests of each age of larvae, as shown in Table 3.

Acute toxicity tests were run in a semi-static system in which the water and the toxicant were fully replaced every day. The volume of each experimental aquarium was 2 L, and the stocking density was 20 individuals per aquarium. No food and no aeration were provided during the experiment, and a photoperiod of 12 h light: 12 h dark was set. An individual Pacific cod larva was considered dead when it lays motionless on the bottom of the aquarium and presented no response to mechanical stimulation for at least 5 sec. The survival status of the larvae was observed twice daily, and the dead individuals were immediately removed.

### Data analysis

The median lethal concentrations (LC\(_{50}\)) for exposure to either ammonia or nitrite during 24, 48, 72, and 96 h periods were estimated by a graphical method using log-probit analysis and indicated as 24-, 48-, 72-, and
96-h LC$_{50}$, respectively. Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s test. These statistical analyses were performed using SPSS 13.0 statistical software, and the significance level was $p < 0.05$ for all analyses.

**Results**

**Ammonia toxicity to Pacific cod larvae**

During the acute toxicity tests, the seawater used had a salinity of 29.8, and the temperature was maintained at 10.0 °C throughout the experiment. The pH was 8.05 ± 0.05 and did not differ among the treatments. No pH adjustment was applied. Three developmental stages of Pacific cod larvae, 11-day post-hatch (wet weight 17.9 ± 0.2 mg and total length 4.63 ± 0.14 mm), 22-day post-hatch (wet weight 33.7 ± 0.5 mg and total length 5.83 ± 0.17 mm), and 35-day post-hatch (wet weight 53.9 ± 1.1 mg and total length 7.46 ± 0.23 mm) were selected for these experiments. No mortalities were observed in the control groups for any of the acute toxicity bioassays performed on different ages of Pacific cod larvae, and none of the test larvae survived the highest exposure concentrations. Figures 1–3 show the percentage mortality for the three different sizes of Pacific cod larvae after 24, 48, 72, and 96 h of exposure to the various concentrations of UIAN, respectively. The mortality levels increased with increasing concentrations of UIAN. Furthermore, Figures 1–3 show that the mortality rate increased with extended exposure durations for each larval age. For example, Figure 3 shows that the mortality rate at a UIAN concentration of 0.39 mg L$^{-1}$ was 47% after 24 h of exposure for the 35-day post-hatch larvae, but increased to 73% after 48 h of exposure, and 93% after 72 h of exposure.

The data from Figures 1–3 were used to calculate the LC$_{50}$ for the different ages of Pacific cod larvae, and these results are summarized in Table 4. For the 11-day post-hatch Pacific cod larvae, the 24- and 48-h LC$_{50}$ for UIAN were 2.88 and 1.72 mg L$^{-1}$, respectively. However, an insufficient number of mortality points for the 11-day post-hatch group precluded calculation of the LC$_{50}$ values for UIAN for 72 and 96 h. For the 22-day post-hatch Pacific cod larvae, the 24-, 48-, 72-, and 96-h LC$_{50}$ values
150  W. WANG ET AL.

for Uian were 0.88, 0.46, 0.45, and 0.36 mg L\(^{-1}\), respectively. For the 35-day post-hatch Pacific cod larvae, the 24-, 48-, 72-, and 96-h LC\(_{50}\) values for Uian were 0.38, 0.32, 0.27, and 0.18 mg L\(^{-1}\), respectively. A one-way ANOVA conducted on the data for the 24-, 48-, 72-, and 96-h LC\(_{50}\) of Uian provided evidence for statistically significant differences (\(p < 0.05\)) among the three developmental stages of Pacific cod larvae. These results clearly demonstrated that the acute toxicity of ammonia is closely related to the age of the Pacific cod larvae, and the acute toxicity of ammonia to the Pacific cod larvae increased with larval growth.

**Nitrite toxicity to Pacific cod larvae**

Under the present experimental conditions, no deaths of individuals were recorded in the control groups during the nitrite acute toxicity tests. The effects of the 24, 48, 72, and 96 h exposures to various concentrations of NO\(_{2}\)-N on the percentage mortality of 11-day post-hatch, 22-day post-hatch, and 35-day post-hatch Pacific cod larvae are shown in Figures 4–6, respectively. Figures 4–6 show that the level of mortality for Pacific cod larvae increased with increasing concentrations of NO\(_{2}\)-N, whereas mortality varied significantly with the age of Pacific cod larvae.

The LC\(_{50}\) values for NO\(_{2}\)-N for different ages of Pacific cod larvae were calculated from the data in Figures 4–6 and are summarized in Table 5. For the 11-day post-hatch Pacific cod larvae, the 24-, 48-, 72-, and 96-h LC\(_{50}\) for NO\(_{2}\)-N were 933.25, 831.76, 726.08, and 304.71 mg L\(^{-1}\), respectively. For the 22-day post-hatch Pacific cod larvae, the 24-, 48-, 72-, and 96-h LC\(_{50}\) of NO\(_{2}\)-N were 354.81, 269.15, 235.54, and 190.55 mg L\(^{-1}\), respectively. Finally, for the 35-day post-hatch Pacific cod larvae, the 24-, 48-, 72-, and 96-h LC\(_{50}\) for NO\(_{2}\)-N were 316.23, 223.87, 189.11, and 116.48 mg L\(^{-1}\), respectively. For the three developmental stages of Pacific cod larvae exposed to nitrite, a one-way ANOVA provided overwhelming evidence of a statistically significant difference in the acute toxicity of nitrite (\(p < 0.05\)). These results illustrated that the acute toxicity of NO\(_{2}\)-N is also related to the age of Pacific cod larvae.

**Discussions**

Because Pacific cod larvae are usually cultured at high densities, the water often accumulates relatively high concentrations of ammonia nitrogen, which originate primarily from the excretions of the cultured organisms and the decomposition of organic matter in water. It is well known that ammonia exists in both ionized and un-ionized forms. The two forms are in equilibrium in water, and the relative proportions of the two forms are dependent on pH, temperature, and salinity. The water quality standards for fisheries assume that the toxicity of ammonia is attributable to the un-ionized form because the un-ionized form of ammonia has no charge and can readily diffuse through epithelial membranes and subsequently cross the blood–brain barrier, resulting in toxicity to the central nervous system. When exposed to un-ionized ammonia, fish show nervous system disturbances, increased gill ventilation, loss of equilibrium, and distressing convulsions. Therefore, in intensive land-based culture systems, ammonia may become a limiting factor with a direct adverse influence on fish growth and survival.

![Figure 3. Percentage mortality of 35-day post-hatch Pacific cod larvae after 24, 48, 72, and 96 h of exposure to increasing concentrations of UIAN.](image)

![Table 4. The lethal concentration (LC\(_{50}\) with 95% confidence limits) of Uian for different stages of Pacific cod larvae.](table)

|              | 11-day post-hatch | 22-day post-hatch | 35-day post-hatch |
|--------------|-------------------|-------------------|-------------------|
| 24-h LC\(_{50}\) (mg L\(^{-1}\)) | 2.88 (2.53–3.13)  | 0.88 (0.68–1.11)  | 0.38 (0.26–0.48)  |
| 48-h LC\(_{50}\) (mg L\(^{-1}\)) | 1.72 (1.42–2.13)  | 0.69 (0.51–0.87)  | 0.32 (0.24–0.43)  |
| 72-h LC\(_{50}\) (mg L\(^{-1}\)) | –                 | 0.45 (0.33–0.64)  | 0.27 (0.20–0.36)  |
| 96-h LC\(_{50}\) (mg L\(^{-1}\)) | –                 | 0.32 (0.21–0.44)  | 0.18 (0.15–0.23)  |
Figure 4. Percentage mortality of 11-day post-hatch Pacific cod larvae after 24, 48, 72, and 96 h of exposure to increasing concentrations of NO$_3^-$-N.

Figure 5. Percentage mortality of 22-day post-hatch Pacific cod larvae after 24, 48, 72, and 96 h of exposure to increasing concentrations of NO$_3^-$-N.

Figure 6. Percentage mortality of 35-day post-hatch Pacific cod larvae after 24, 48, 72, and 96 h of exposure to increasing concentrations of NO$_3^-$-N.

Table 5. The lethal concentration (LC$_{50}$ with 95% confidence limits) of NO$_3^-$-N to different stages of Pacific cod larvae.

|                | 11-day post-hatch | 22-day post-hatch | 35-day post-hatch |
|----------------|-------------------|-------------------|-------------------|
| 24-h LC$_{50}$ (mg L$^{-1}$) | 933.25 (873.31–997.14) | 354.81 (286.53–411.27) | 316.23 (257.53–372.15) |
| 48-h LC$_{50}$ (mg L$^{-1}$) | 831.76 (790.17–873.25) | 269.15 (255.45–282.60) | 223.87 (212.68–235.06) |
| 72-h LC$_{50}$ (mg L$^{-1}$) | 726.08 (768.77–762.30) | 235.54 (223.76–247.32) | 189.11 (179.65–198.57) |
| 96-h LC$_{50}$ (mg L$^{-1}$) | 304.71 (289.31–321.08) | 190.55 (181.02–201.03) | 116.48 (109.67–123.30) |
In this study, the acute toxicity of ammonia was tested on different ages of Pacific cod larvae, and the median lethal concentrations of un-ionized ammonia varied with larval ages. For example, the 24-h LC₅₀ values for UIAN to 11-day post-hatch and 35-day post-hatch larvae were 2.88 and 0.38 mg L⁻¹, respectively. On the other hand, it is important to note that the lowest values of the 96-h LC₅₀ that produced acute toxic symptoms in post-hatch Pacific cod larvae were observed to be approximately 0.18 mg L⁻¹ under the present experimental conditions.

It is difficult to directly compare the present results with the acute ammonia toxicity values reported for other species, because they are dependent on environmental conditions and on the biological characteristics of fish. However, the data obtained in this study on 24- to 96-h LC₅₀ values were within the range of values reported in other species.[28,29] In general, saltwater species of fish are slightly more sensitive to ammonia toxicity than freshwater species. The averages of the mean acute toxicity values of UIAN for 32 freshwater species and 17 saltwater species reported by Randall and Tsui [30] were 2.79 and 1.86 mg L⁻¹, respectively. Handy and Poxton [31] indicated that the un-ionized form of ambient ammonia is acutely toxic to marine fish in the range of 0.1–3.4 mg L⁻¹. Barimo and Walsh [32] investigated the effects of acute ammonia exposure during the early life stages of the gulf toadfish (Opsanus beta). They reported that the embryos had a higher 96-h LC₅₀ value than the larvae: the LC₅₀ values were 29.08 and 2.80 mg L⁻¹ for embryos and larvae, respectively. Person-Le et al. [28] studied acute ammonia toxicity in sea bass (Dicentrarchus labrax), sea bream (Sparus aurata), and turbot (Scophthalmus maximus) juveniles weighing from 6 to 163 g under optimal environmental conditions (17–18 °C, salinity 34, pH 8.15, and oxygen over 75% saturation). These investigators found that the 96-h LC₅₀ for UIAN averaged 1.7 mg L⁻¹ in sea bass, compared with 2.5–2.6 mg L⁻¹ in the sea bream and turbot. Other reports for LC₅₀ values at 96 h in sea bream juveniles weighing between 0.4 and 3 g were 1.27 mg L⁻¹.[33] Øelsrud and Pearson [34] studied the lethal effects of ammonia toxicity to juvenile barramundi (Lates calcarifer) (weight 1.25 ± 0.009 g and length 48.08 ± 0.14 mm) and found that the 24-, 48-, and 96-h LC₅₀ values for UIAN in barramundi were 1.59, 1.47, and 1.3 mg L⁻¹, respectively. Rodrigues et al. [35] evaluated the acute toxicity of ammonia to juvenile cobia (Rachycentron canadum) (weight 1.74 ± 0.11 g) at a test temperature of 26 °C and a salinity of 22 and obtained a 96-h LC₅₀ value for UIAN of 1.13 mg L⁻¹. Miller et al. [36] found that the 96-h LC₅₀ for UIAN in larval inland silversides (Menidia beryllina) was 0.97 mg L⁻¹ at 25 °C, 0.71 mg L⁻¹ at 31 °C, and pH 7. Piedras et al. [37] stated that the 96-h LC₅₀ for UIAN in newly hatched pejerrey (Odontesthes bonariensis) larvae (average weight 3.9 mg) was 0.71 mg L⁻¹. Costa et al. [38] reported that the 96-h LC₅₀ values for UIAN in juvenile pompano (Trachinotus marginatus) (0.86 ± 0.21 g) were 0.66, 1.87, and 1.06 mg L⁻¹ at salinity levels of 5, 10, and 30, respectively. Barbieri and Doi [39] also used juvenile cobia (Rachycentron canadum) with total lengths of 15.16 ± 0.92 cm and weights of 19.26 ± 4.5 g as test subjects at 25 °C, and they found that the 96-h LC₅₀ value for UIAN was 0.68 mg L⁻¹ at a salinity of 35, 0.65 mg L⁻¹ at a salinity of 20, and 0.31 mg L⁻¹ at a salinity of 5, respectively. These values are similar to those reported by Weirich and Riche [40] in a study of the acute toxicity of UIAN to juvenile black sea bass (Centropristis striata) (mean weight 9.9 g), in which the 96-h LC₅₀ values ranged from 0.46 to 0.54 mg L⁻¹. Similar results were reported by Miron et al. [22], who determined that the 96-h LC₅₀ values for UIAN in silver catfish (Rhamdia quelen) were 0.44, 1.45, and 2.09 mg L⁻¹ at pH 6.0, 7.5, and 8.2, respectively.

To the best of our knowledge, this study represents the first attempt to compare acute ammonia toxicity in different ages of Pacific cod larvae. This result indicates that the acute toxicity of ammonia is clearly related to the age of Pacific cod larvae. The age-dependent sensitivity of Pacific cod larvae to ammonia may be associated with the observed continuous tendency for the overall survival rate of the larvae to decrease during the entire artificial rearing process. Therefore, a better understanding of the interactions between Pacific cod larval growth and water quality could contribute to enhancing the production of the larvae. In comparison, the observations of Barimo and Walsh [32] showed that the early life stages of the gulf toadfish (Opsanus beta) were more tolerant towards ammonia than either juveniles or adults. Their study also confirmed that the LC₅₀ values were at least two orders of magnitude greater than the concentrations that naturally occur at nest sites.[33] Adelman and coworkers [41] also reported that at a pH of 8.0 and a temperature of 25 °C, adult topeka shiners (Nototropis Topeka) were more sensitive to ammonia than juveniles. However, several investigators have observed that the sensitivity of some species increased with growth. For example, Zhang and coworkers [42] conducted 96-h LC₅₀ tests using four different sizes of yellow catfish (Peleobagrus fulvidraco) to provide primary information on the sensitivity of this species to the toxicity of ammonia and found that 96-h LC₅₀ of fish weighing 0.034, 0.296, 3.52, and 32.96 g to UIAN were 0.34, 0.49, 0.65, and 0.94 mg L⁻¹, respectively, indicating that the susceptibility of this fish to ammonia decreased with increasing size. Gomulka and coworkers [43] found that the susceptibility of juvenile chub (Leuciscus cephalus) to acute ammonia toxicity decreased linearly with age. In this study, the 48-h LC₅₀ values ranged from 0.62 mg L⁻¹ of UIAN for one day after first feeding larvae to 1.73 mg L⁻¹ for those 30 days after first feeding.

This study also provides data concerning the acute toxicity of exposure to various concentrations of NO₂⁻-N
in Pacific cod larvae. The 96-h LC50 of NO2-N to 11-day post-hatch, 22-day post-hatch, and 35-day post-hatch Pacific cod larvae are 304.71, 190.55, and 116.48 mg L\(^{-1}\), respectively. It has been reported that the acute toxicity of nitrite to fish differs according to the fish species. Rodrigues et al. [35] evaluated the acute toxicity of nitrite to juvenile cobia (O. canadum) (weight 0.88 ± 0.06 g) and found that only 30% of the individuals died at a concentration of 210 mg L\(^{-1}\) NO2-N after 96-h exposure. Weirich and Riche [40] studied the acute toxicity of nitrite to juvenile black sea bass (C. striata) (mean weight 9.9 g). The reported LC50 values for NO2-N in the fish exposed for 96-h ranged from 190.0 to 241.9 mg L\(^{-1}\). These results indicate that the juvenile black sea bass are highly resistant to NO2-N exposure. Costa et al. [38] reported that the 96-h LC50 values for exposure of juvenile pompano (T. marginatus) (0.86 ± 0.21 g) to NO2-N were 39.94, 116.68, and 37.55 mg L\(^{-1}\) at salinity levels of 5, 10, and 30, respectively. Doleželová et al. [25] determined that the 96-h LC50 value for NO2-N in zebra fish (D. rerio) was 242.6 mg L\(^{-1}\). Zhang and coworkers [42] studied the toxicity of nitrite to four different sizes of yellow catfish (Pelteobagrus fulvidraco) and showed that the 96-h LC50 values for NO2-N in the fish exposed for 2–3 months. Adelman and coworkers [41] obtained the same conclusion: in adult and juvenile topeka shiners (N. Topeka), the 96-h LC50 for NO2-N were 6.1 and 8.3 mg L\(^{-1}\), respectively. However, some authors have reported that smaller fish are more sensitive to nitrite than bigger ones. [45] For example, Alcaraz and Espina [46] studied the acute toxicity of nitrite in juvenile grass carp (Ctenopharyngodon idella) and found that the smaller fish were more sensitive than the larger fish, with 96-h LC50 values for the former group being 3.4 times higher than the latter group.

Conclusions
The results obtained in this study show the acute toxicity of ammonia and nitrite to selected ages of Pacific cod larvae. These data are important for water quality control during the larval rearing period. Furthermore, the results clearly demonstrate that the acute toxicity of ammonia is closely related to the age of Pacific cod larvae. It can be assumed that the days following hatching are the most critical period for the Pacific cod larvae because of their sensitivity to ammonia. Thus, during that period, special attention should be paid to water quality monitoring in intensive rearing tanks.

Acknowledgments
We greatly appreciate the support of the National Nature Science Foundation of China (41101481, 21107019). This work was also supported by the Public Subject of the Committee of Science and Technology of Liaoning province of China (2012005001), the Science and Technology Foundation of Education Department of Liaoning Province of China (L2011117), and the Natural Science Foundation of Liaoning Province of China (2014202149).

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
National Nature Science Foundation of China (41101481, 21107019). This work was also supported by the Public Subject of the Committee of Science and Technology of Liaoning province of China (2012005001), the Science and Technology Foundation of Education Department of Liaoning Province of China (L2011117), and the Natural Science Foundation of Liaoning Province of China (2014202149).

Notes on contributors
Wei Wang associate professor of Dalian Ocean University, has published more than 20 articles. His main research area is aquaculture.
Hua Wang associate professor of Dalian Ocean University, has published more than 30 articles. His main research area is aquatic chemistry of aquaculture.
Chunyan Yu associate professor of State Oceanic Administration, has published more than 15 articles. His main research area is environmental chemistry.
Zhigiang Jian professor of Dalian Ocean University, has published more than 50 articles. His main research area is aquaculture.

References
[1] Smith RL, Paul AJ, Paul JM. Seasonal changes in energy and the energy cost of spawning in Gulf of Alaska Pacific cod. J. Fish. Biol. 1990;36:307–316.
[2] Chung S, Kim S, Kang S. Ecological relationship between environmental factors and Pacific cod (Gadus macrocephalus) catch in the southern East/Japan Sea. Anim. Cells. Syst. 2013;17:374–382.
[3] Bigg GR. Environmental confirmation of multiple ice age refugia for Pacific cod, Gadus macrocephalus. Evol. Ecol. 2014;28:177–191.
[4] Davis MW, Ottmar ML. Vertical distribution of juvenile Pacific cod Gadus macrocephalus: potential role of light, temperature, food, and age. Aquat. Biol. 2009;8:29–37.
[5] Liu M, Lu ZC, Gao TX, et al. Remarkably low mtDNA control-region diversity and shallow population
structure in Pacific cod Gadus macrocephalus. J. Fish. Biol. 2010;77:1071–1082.
[6] Ottmar ML, Hurst TP. Thermal effects on swimming activity and habitat choice in juvenile Pacific cod (Gadus macrocephalus). Mar. Biol. 2012;159:2185–2194.
[7] DiMaria RA, Miller JA, Hurst TP. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod Gadus macrocephalus. Environ. Boil. Fish. 2010;89:453–462.
[8] Hurst TP, Cooper DW, Scheingross JS, et al. Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod (Gadus macrocephalus). Fish. Oceanogr. 2009;18:301–311.
[9] Laurel BJ, Copeman LA, Parrish CC. Role of temperature on lipid/fatty acid composition in Pacific cod (Gadus macrocephalus) eggs and unfed larvae. Mar. biol. 2012;159:2025–2034.
[10] Holan AB, Wold PA, Leiknes TO. Intensive rearing of cod larvae (Gadus morhua) in recirculating aquaculture systems (RAS) implementing a membrane bioreactor (MBR) for enhanced colloidal particle and fine suspended solids removal. Aquacult. Eng. 2014;58:52–58.
[11] Boudreax PJ, Ferrara AM, Fontenot QC. Acute toxicity of ammonia to spotted gar, Lepisosteus oculatus, alligator gar, Atractosteus spatula, and paddlefish, Polyodon spathula. J. World. Aquacult. Soc. 2007;38:322–325.
[12] Feyjoo P, Riera R, Felipe BC, et al. Tolerance response to ammonia and nitrite in hatchlings paralarvae of Octopus vulgaris and its toxic effects on prey consumption rate and chromatophores activity. Aquacult. Int. 2011;19:193–204.
[13] Foss A, Kristensen T, Atland A, et al. Effects of water reuse on lipid/fatty acid composition in Pacific cod (Gadus morhua) eggs and unfed larvae. Mar. biol. 2012;159:2025–2034.
[14] Person-Le RJ, Chartois H, Quemener L. Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture. 1995;136:181–194.
[15] Rodrigues RV, Schwarz MH, Delbos BC, et al. Acute toxicity and sublethal effects of ammonia and nitrite for juvenile cobia Rachycentron canadum. Aquaculture. 2007;271:244–251.
[16] Foss A, Siikavuopio SI, Sæther B, et al. Effect of chronic ammonia exposure on growth in juvenile Atlantic cod. Aquaculture. 2004;237:179–189.
[17] Person-Le RJ, Chartois H, Quemener L. Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture. 1995;136:181–194.
[18] Foss A, Siikavuopio SI, Sæther B, et al. Effect of chronic ammonia exposure on growth in juvenile Atlantic cod. Aquaculture. 2004;237:179–189.
[19] Foss A, Imsland AK, Roth B, et al. Interactive effects of oxygen saturation and ammonia on growth and blood physiology in juvenile turbot. Aquaculture. 2007;271:244–251.
[20] Doleželová P, Máčková S, Pištěková V, et al. Nitrite toxicity assessment in Danio rerio and Poecilia reticulata. Acta. Vet. Brno. 2011;80:309–312.
[21] Jensen FB. Review: nitrite disrupts multiple physiological functions in aquatic animals. Comp. Biochem. Phys. A. 2003;135:9–24.
[22] Voslárová E, Pištěková V, Svobodová Z. Nitrite toxicity to Danio Rerio: effects of fish age and chloride concentrations. Acta. Vet. Brno. 2006;75:107–113.
[23] Person-Le RJ, Chartois H, Quemener L. Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture. 1995;136:181–194.
[43] Gomułka P, Zarski D, Kucharczyk D, et al. Acute ammonia toxicity during early ontogeny of chub, Leuciscus cephalus (Cyprinidae). Aquat. Living Resour. 2011;24:211–217.
[44] Voslárová E, Pištěková V, Svobodová Z, et al. Nitrite toxicity to Danio rerio: effects of subchronic exposure to fish growth. Acta Vet. Brno. 2008;77:455–460.
[45] Almendras JME. Acute toxicity and methemoglobinemia in juvenile milkfish (Chanos chanos Forsskae). Aquaculture. 1987;61:33–40.
[46] Alcaraz G, Espina S. Acute toxicity of nitrite in juvenile grass carp modified by weight and temperature. Bull. Environ. Contam. Toxicol. 1995;55:473–478.