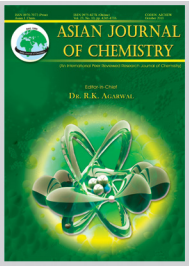
Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5309-5312

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



# Fatty Acid Composition of Chironomidae Larvae in Different Seasons

TUBA KARA*, AYDA TELLIOGLU and SEVINC AYDIN

Department of Biology, Faculty of Science, Firat University, 23169 Elazig, Turkey

*Corresponding author: E-mail: atellioglu69@gmail.com

(*Received*: 1 November 2011; *Accepted*: 16 June 2012) AJC-11624

In the present study, seasonal changes in the fatty acid composition of the chironomidae larvae were determined by gas chromatography. When the seasonal variation of fatty acids were compared, a significant increase were observed caproic acid (6:0), palmitic acid (16:0), palmitoleic acid (16:1 n7), stearic acid (18:0), oleic acid (18:1 n9), linoleic acid (18:2 n6) and arachidonic acid (20:4 n6) in all seasons. A significant increase were observed in myristic acid (14:0) in spring and winter; margaroleic acid (17:1) in autumn; eicosapentaenoic acid in spring and linolenic acid (18:3 n3) in spring, summer and winter. When the fatty acids were compared, caproic acid (6:0), palmitic acid (16:0), stearic acid (18:0) and linoleic acid (18:2 n6) level were found to be significantly high. When fatty acids were compared statistically, differences were detected between seasons ($p < 0,001$). In conclusion, it is suggested that the reason for these differences is due to the variety of food in different seasons and larval development feature.

**Key Words: Chironomidae larvae, Fatty acids, Seasonal variation.**

## INTRODUCTION

Several factors have been shown to affect chironomidae larval growth: temperature, quality and quantity of food, intra-and interspesific competition for food resources and the risk of predation[1-6]. Under field conditions, larval growth and development can reportedly be influenced by the combination of the above factors and others such as oxygen, pH, toxic substances, discharges *etc*.[7].

Fatty acids serve various functions in insects. They are the primary energy source during periods of nonfeeding, such as diapause and as structural components of membranes. Fatty acid compositions are not fixed in insects and can change seasonally to perform special functions that may be critical for survival. In particular, development[8,9], diet[9-16] and diapauses status[17-21] exert strong influences on the shape of fatty acid profiles.

Fish and aquatic invertebrates are one of the main sources of the polyunsaturated fatty acid (PUFA) in human nutrition, therefore, aquatic ecosystems are considered as an important resource of essential PUFA's[22]. Such findings have resulted in the suggestion that researches should attempt to measure the total yield of PUFA in aquatic ecosystems[23]. Benthic invertebrates (zoobenthos) are one of the most important components of riverine ecosystems and a major food resource for fish. There are a number of reports of fatty acid composition and content in freshwater zoobenthos in lakes, ponds and streams[23-27], whereas such data for large rivers are scarce. But recently, the river ecosystem, taxonomic groups in the fatty acid compounds of the studies showed an increase[26,28].

The aim of this work is to analyze of fatty acid content of chironomidae larvae and then statistically compare the variation between seasons.

## EXPERIMENTAL

Chironomid larvae were collected on spring (April), summer (July), autumn (October) and winter (December) 2010 in Büyük Stream (Pelte/Elazig). Then samples were stored at deep-freeze until analysis.

**Extraction of lipids:** The weights of samples were weighed and then were extracted with hexane/isopropyl alcohol (3:2 v/v)[29]. The lipid extracts were centrifuged at 5,000 rpm for 5 min at 4 ºC.

**Fatty acid analyses:** Fatty acid in the lipid extracts were converted into methyl esters by means of 2 % sulphuric acid (v/v) in methanol[30]. The fatty acid methyl esters were extracted with *n*-hexane. Then the methyl esters separated and quantified by gas chromatography and flame ionization detection (Shimadzu GC, 17 Ver.3) coupled to a class GC 10 software computing recorder. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Machery-Nagel, Germany) using nitrogen as carrier gas (flow rate 0.8 mL/min) the temperatures of the column, detector and injector valve were 120-220, 240, 280 ºC, respectively. Identification of the individual methyl esters was

TABLE-1
FATTY ACID COMPOSITION OF CHIRONOMIDAE LARVAE IN DIFFERENT SEASONS (mg/g)

| Fatty acids | Spring | Summer | Autumn | Winter |
|---|---|---|---|---|
| Caproic acid 6:0 | 13.86 ± 0.13 | 14.44 ± 0.009 | 17.5 ± 0.52[d] | 14.5 ± 0.41 |
| Myristic acid 14:0 | 1.67 ± 0.004 | 1.10 ± 0.003 | 1.20 ± 0.003 | 1.95 ± 0.005[d] |
| Palmitic acid 16:0 | 15.54 ± 0.45 | 15.6 ± 0.11 | 20.35 ± 0.84[d] | 15.83 ± 0.40 |
| Palmitoleic acid 16:1 n7 | 4.72 ± 0.009 | 5.45 ± 0.009[d] | 3.05 ± 0.009 | 4.27 ± 0.005 |
| Margaroleic acid 17:1 | 1.10 ± 0.003 | 1.10 ± 0.003 | 1.94 ± 0.004[d] | 1.20 ± 0.003 |
| Stearic acid 18:0 | 12.11 ± 0.36 | 14.62 ± 0.007 | 17.85 ± 0.52[d] | 12.13 ± 0.12 |
| Oleic acid 18:1 n9 | 12.64 ± 0.009 | 26.25 ± 0.52[d] | 16.68 ± 0.10 | 21.43 ± 0.25 |
| Linoleic acid 18:2 n6 | 25.54 ± 0.43[d] | 15.37 ± 0.15 | 19.41 ± 0.41 | 20.01 ± 0.005 |
| Linolenic acid 18:3 n3 | 2.07 ± 0.008 | 2.15 ± 0.004 | 1.14 ± 0.004 | 2.28 ± 0.005[b] |
| Arachidonic acid 20:4 n6 | 3.38 ± 0.008 | 3.68 ± 0.15 | 4.92 ± 0.11[d] | 3.43 ± 0.008 |
| Eicosapentaenoic acid 20:5 n3 | 3.25 ± 0.005[d] | 2.41 ± 0.008 | 1.10 ± 0.004 | 2.80 ± 0.004 |

d: $p < 0.001$ c: $p < 0.01$ b: $p < 0.05$ a: $p > 0.05$

performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions[31].

**Statistical analysis:** Statistical analysis was performed using SPSS software (ver. 10.0). The experimental results were reported as mean ±SEM (standard error of means). Analysis of variance (ANOVA) and an LSD (least significant difference) test were used to compare the experimental groups.

## RESULTS AND DISCUSSION

When the fatty acids were compared, oleic acid (18:1 n9), linoleic acid (18:2 n6), palmitic acid (16:0) and stearic acid (18:0) level were found to be significantly high among fatty acids ($p < 0.001$) (Table-1).

Saturated fatty acid of caproic acid (6:0), palmitic acid (16:0) and stearic acid (18:0) were found high level in all seasons. Unsaturated fatty acid of oleic acid (18:1 n9) and linoleic acid (18:2 n6) were detected high level in present study (Table-1).

When the seasonal variation of fatty acid content were compared, a significant increase were observed in linoleic acid (18:2 n6) and eicosapentaenoic acid (EPA; 20:5 n3) in spring ($p < 0.001$). A significant increase were observed in palmitoleic acid (16:1 n7) and oleic acid (18:1 n9) in summer ($p < 0.001$) (Fig. 1).

Caproic acid (6:0), palmitic acid (16:0), margaroleic acid (17:1), stearic acid (18:0) and arachidonic acid (20:4 n6) level were found to be significantly high in autumn ($p < 0.001$). When the fatty acids were compared, a significant increase was observed in myristic acid (14:0) and a partial increase was observed in linolenic acid (18:3 n3) in winter ($p < 0.001$, $p < 0.05$) (Fig. 1).

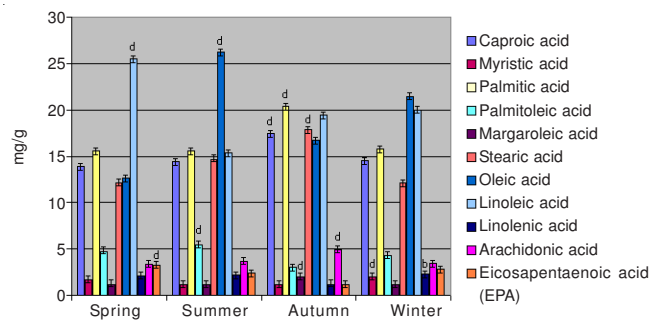



Fig. 1. Levels of fatty acids in chironomidae larvae (mg/g); d: $p < 0.001$ c: $p < 0.01$ b:$p < 0.05$ a: $p > 0.05$

When the seasonal variation of polyunsaturated fatty acids compound levels were compared, linoleic acid (18:2 n6) was found high level in spring ($p < 0.001$) (Fig. 2). There was no significant difference in summer. Linolenic acid, arachidonic acid and eicosapentaenoic acid were found low level in all seasons (Fig. 2).

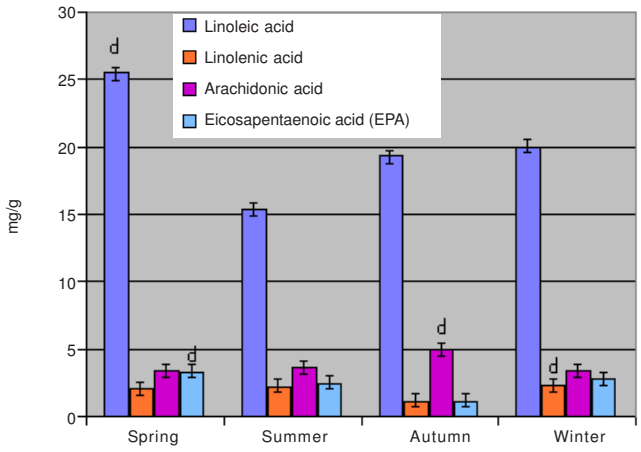



Fig. 2. Level of PUFA (Polyunsaturated fatty acid) in chironomidae larvae (mg/g); d: $p < 0.001$ c: $p < 0.01$ b : $p < 0.05$ a: $p > 0.05$

In this study stearic acid, oleic acid, palmitic acid and linoleic acid were found significantly high (Table-1). This situation is properly with chironomidae larvae and other insects[32-34]. Some researches determined fatty acid in insect larvae. They found oleic acid and palmitic acid high level[35,36]. Similarly, oleic acid and palmitic acid level were found significantly high in our study (Table-1).

Insects can modify their fatty acid composition in response to changes in environmental conditions. The ability to elongate and desaturate tissue polyunsaturated fatty acids is one of the mechanisms of changing fatty acid profiles[37]. Here, we note differences in fatty acid composition of chironomidae larvae in different seasons. Linoleic acid has been determined to be essential to many insects belonging to various orders[38]. Some species of insects were shown to be able to biosynthesize linoleic acid[39-42]. One of the major functions of linoleic acid and polyunsaturated in general, is as a structural component of membranes to maintain proper fluidity and permeability. In addition, increased proportions of unsaturated fatty acids

in triglycerides of poikilothermic organism have been often interpreted as an adaptation to cold, related to the maintenance of an appropriate fluidity of the depot lipids to make them available as energy resources[43-47].

Food affects fatty acid composition in insects[48]. Especially chironomidae larvae are fed plant-derived foods in spring and summer. So that palmitoleic acid, oleic acid, linoleic acid and eicosapentaenoic acid were found significantly higher in spring and summer than in autumn and winter (Fig. 1). Eicosatrienoic acid (20:3 n6), arachidonic acid (20:4 n6) and eicosapentaenoic acid (EPA) (20:5 n3) such as 20 carbon polyunsaturated fatty acids are most abundant in aquatic insects[48]. In our study these fatty acid were detected. But these fatty acids penetrate cell membrane and store in phospholipids structure[49-52], so these fatty acids were found lower level in chironomidae larvae (Table-1). In addition, were detected that 20 carbon polyunsaturated fatty acids contribute to the formation of cellular immunity against the bacteria[49,53]. Also were detected that fatty acids play role to formation of body temperature[54-56]. In our study example of this situation, these fatty acids were determined in spring and autumn in Büyük Stream (Fig. 2).

Food quality may be more important than food quantity for survival, growth and reproduction in animal populations. Evidence for this comes primarily from zooplankton studies[57-59], but also from studies with benthic macroinvertebrates[60-62] and microinvertebrates[63] and fish[64]. These studies use the contents of long-chain (≥ 20 C) polyunsaturated fatty acids of the linolenic (ω3) and linoleic (ω6) families, for example eicosapentaenoic acid (20:5 ω3) and docosahexaenoic acid (22:6 ω3), as indicators of food quality[25].

Certain fatty acids from dietary sources are conservatively transferred into neutral lipids and used as organic markers in studies of trophic relationships[65-67]. For example, palmitoleic acid (16:1 n7) or the ratio between the sum of desaturated 16C fatty acids relative to 16:0 is indicative of diatoms[67], whereas the branched *iso*- and *anteiso*- forms of 15C and 17C fatty acids indicate a bacterial origin[68].

Fatty acid biomarkers showed that larvae of the autumn cohort of *Chironomus plumosus* were closely associated with the detrial food chain during spring and summer, supporting the conjecture that these larvae feed on low-quality food. Probably the smaller *Chironomus plumosus* larvae are ineffective at irrigating their tubes (inefficient filter-feeders) and may have been feeding mainly on microflora associated with the tube walls or in deeper sediment strata, instead of utilizing diatoms as a food resource[25]. These results support our study that these findings as observed in chironomidae larvae. Lots of fatty acid was found high level in autumn and winter (Table-1).

Insects are similar fatty acid content of qualitative, but there are differences in terms of quantitative. For example, in Diptera palmitoleic acid extremely high when is compared other fatty acid groups[33,48,69]. In our study was detected palmitoleic acid composition in chironomidae larvae extremely high level in summer. The reason for this situation, larvae feed plant-derived food in summer. Phytoplankton algae are the primary source of larval food for some chironomid species[70,71] and hence an important factor that influences the population dynamics of these midges[71-73].

Suschik and others[28], studied fatty acids components of benthic invertebrates in the Yenisei river, they determined miristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) high level in chironomidae larvae. They suggested that these data indicate marked part of chironomid diet in Yenisei river comprized diatoms. In our study, stearic acid and palmitic acid were found significantly high in Büyük stream. In addition, Suschik and others[28], determined margaroleic acid (17:1), linolenic acid (18:3 n3), arachidonic acid (20:4 n6) and eicosapentaenoic acid (20:5 n3) low lever in chironomidae larvae. Similarly, in our study, we detected these fatty acids low lever in all seasons (Table-1).

Kiyasho and others[34] studied *Stictochironomus pictulus* (chironomidae) fatty acid component and they detected palmitic acid and palmitoleic acid were high level. Khani and others[37], detected lipid and fatty acid composition of *Cydia pomonella* larvae and they found palmitic acid and linoleic acid level were significantly high level. Similarly, our study, we detected these fatty acids high level.

The difference between seasons cause to difference in daily activities and metabolism of insect in different photoperiod conditions. Close to each other in daily illumination time, were closer to the amount of lipid. Photoperiod is due to make similar effect to nutrition, reproduction and metabolism. Different researches suggested that very small changes in photoperiod can make important differences[74]. Quality and quantity of food affect larvae. Seasonal condition plays role fatty acid composition of chironomidae larvae.

In conclusion, we determined seasonal variation of fatty acids composition of chironomidae larvae. We have detected difference in fatty acids content between seasons. As the cause of this situation, it is suggested that these differences are due to the variety of food and larval development feature in different seasons. Our findings and our research is new for Turkey. Therefore, these findings are important for biochemical analysis of future.

## ACKNOWLEDGEMENTS

This work has been supported by the Firat University Scientific Research Project Unit (FUBAP Project number FF.11.08).

## REFERENCES

1. G.M. Ward and K.W. Cummins, *Ecology*, **60**, 57 (1979).
2. M. Ladle, J.S. Welton and J.A.B. Bass, *Arch. Hydrobiol*., **102**, 201 (1984).
3. R.L. Mattingly, K.W. Cummins and R.H. King, *Hydrobiologia*, **77**, 161 (1981).
4. S.L. Ball and R.L. Baker, *Freshwater Biol*., **34**, 1 (1995).
5. Z. Kajak, *Ekol. Pol. (Series A)*, **11**, 355 (1963).
6. T. Iwakuma, Ph. D. Thesis, Kyushu University, Japan (1986).
7. M. Tokeshi, in eds.: P.D. Armitage, P.S. Cranston and L.C.V. Pinder, Life cycles and population dynamics. The Chironomidae: The Biology and Ecology of Non-biting Midges. Chapman and Hall, London, U.K., pp. 224-268 (1995).
8. M. Bashan, H. Akbas and K. Yurdakoc, *Comp. Biochem. Physiol. B*, **132**, 375 (2002).
9. K. Bozkus, *Turk. J. Biol.*, **27**, 73 (2003).
10. G.C. Rock, R.L. Patton and E.H. Glass, *J. Insect. Physiol.*, **11**, 91 (1965).
11. S. Turunen, *J. Insect Physiol.*, **20**, 1257 (1974).
12. J.W. Barnett and R.S. Berger, *Ann. Entomol. Soc. Am.*, **63**, 917 (1970).
13. P.A. Grau and L.C. Terriere, *J. Insect. Physiol.*, **17**, 1637 (1971).

14. N.W. Earle, B. Slaten and M.L. Jr. Burks, *J. Insect. Physiol.*, **13**, 187 (1967).
15. D.W. Stanley-Samuelson and R.H. Dadd, *J. Insect. Physiol.*, **27**, 571 (1981).
16. D.W. Stanley-Samuelson, E.W. Rapport and R.H. Dadd, *Comp. Biochem. Physiol. B*, **81**, 749 (1985).
17. S.M. Valder, T.L. Hopkins and S.A. Valder, *J. Insect Physiol.*, **15**, 1199 (1969).
18. M. Azuma, Y. Yoshimi and T. Furusawa, *Appl. Entomol. Zool.*, **24**, 180 (1989).
19. I. Shimizu, *Comp. Biochem. Physiol. B*, **102**, 713 (1992).
20. M. Hodková, P. Simek, H.A. Zahradnícková and O. Nováková, *Insect Biochem. Molec. Biol.*, **29**, 367 (1999).
21. M. Bashan and O. Cakmak, *Ann. Entomol. Soc. Am.*, **98**, 575 (2005).
22. W. Steffens, *Aquaculture*, **151**, 97 (1997).
23. M.T. Arts, R.G. Ackman and B.J. Holub, *Can. J. Fish. Aquat. Sci.*, **58**, 122 (2001).
24. C. Ghioni, J.C. Bell and J.R. Sargent, *Comp. Biochem. Physiol. B*, **114**, 161 (1996).
25. W. Goedkoop, L. Sonetsen, H. Markensten and G. Ahlgren, *Freshwater Biol.*, **40**, 135 (1998).
26. W. Goedkoop, L. Sonetsen, G. Ahlgren and M. Boberg, *Can. J. Fish. Aquat. Sci.*, **57**, 2267 (2000).
27. M.I. Gladyshev, N.N. Sushchik, G.N. Skoptsova, L.S. Parfentsova and G.S. Kalachova, *Dokl. Akad. Nauk*, **364**, 566 (1999).
28. N.N. Sushchik, M.I. Gladyshev, A.V. Moskvichova, O.N. Makhutova and G.S. Kalachova, *Comp. Biochem. Physiol. B*, **134**, 111 (2003).
29. A. Hara and N.S. Radin, *Anal. Biochem.*, **90**, 420 (1978).
30. W.W. Christie, Gas Chromatography and Lipids, The Oil Press, Glasgow (1990).
31. S. Celik, H. Akarcay, O. Yilmaz and A. Ozkaya, *Cell Bioc. Funct.*, **24**, 561 (2006).
32. D.W. Stanley-Samuelson and R.H. Dadd, *Insect Biochem.*, **13**, 549 (1983).
33. S.N. Thompson, *Comp. Biochem. Physiol. B*, **45**, 467 (1973).
34. S.I. Kiyashko, A.B. Imbs, T. Narita, V.I. Svetashev and E. Wada, *Comp. Biochem. Physiol. B*, **139**, 705 (2004).
35. A. Aktumsek and M.Y. Aksoylar, *Doga TU J. Biol.*, **11**, 10 (1987).
36. A. Aktumsek, Z.U. Nurullahoglu and L. Kalyoncu, *Selcuk Uni. J. Sci and Art Fac.*, **17**, 29 (2000).
37. A. Khani, S. Moharramipour, M. Bargezar and H. Naderi-Manesh, *Insect. Sci.*, **14**, 125 (2007).
38. L.E. Canavoso, Z.E. Jouni, K.J. Karnas, J.E. Pennington and M.A. Wells, *Ann. Rev. Nut.*, **21**, 23 (2001).
39. L.A. Dwyer and G.J. Blomquist, *Lipid Res.*, **20**, 215 (1981).
40. C. Cripps, G.J. Blomquist and D.M. Renobales, *Biochim. Biophys. Acta*, **876**, 572 (1986).
41. D.M. Renobales, C. Cripps, D.W. Stanley-Samuelson, R.A. Jurenkaa and G.J. Blomquist, *Trends Biochem. Sci.*, **12**, 364 (1987).
42. G.J. Blomquist, C.E. Borgeson and M. Vundla, *Insect. Biochem.*, **21**, 99 (1991).
43. J.R. Hazel, *J. Exp. Zool.*, **207**, 33 (1979).
44. C.I. Frank, *Can. J. Zool.*, **69**, 2707 (1991).
45. T. Ohtsu, C. Katagiri, M.T. Kimura and S.H. Hori, *J. Biol. Chem.*, **268**, 1830 (1993).
46. D.R. Joanisse and K.B. Storey, *Physiol. Zool.*, **69**, 1079 (1996).
47. R. Kakela and H. Hyvarien, *Comp. Biochem. Physiol. B*, **115**, 501 (1996).
48. D.W. Stanley-Samuelson, R.A. Jurenka, C. Cripps, G.J. Blomquist and M. de Renobales, *Arch. Insect. Biochem. Physiol.*, **9**, 1 (1988).
49. J.S. Miller, R.W. Howard, A. Nguyen, R.M.T. Rosario and D.W. Stanley-Samuelson, *Proc. Nat. Acad. Sci. (USA)*, **88**, 1064 (1991).
50. D.W. Stanley-Samuelson, *Comp. Biochem. Physiol. B*, **85**, 369 (1986).
51. C.L. Ogg and D.W. Stanley-Samuelson, *Comp. Biochem. Physiol. B*, **101**, 345 (1992).
52. C.L. Ogg, L.J. Meinkle, R.W. Howard and D.W. Stanley-Samuelson, *Comp. Biochem. Physiol. B*, **105**, 69 (1993).
53. E.V. Kerkhove, P. Pirotte, D.H. Petzel and D.W. Stanley-Samuelson, *J. Insect. Physiol.*, **41**, 435 (1994).
54. D.W. Stanley, Eicosanoids in Invertebrate Signal Transduction Systems, Princeton University Press, Princeton, N.J., p. 277 (2000).
55. D.W. Stanley, A.R. Nor Aliza, H. Tunaz, S.M. Putnam, Y. Park and J.C. Bedick, *Neotrop. Entomol.*, **31**, 341 (2002).
56. D.W. Stanley, *Annu. Rev. Entomol.*, **51**, 25 (2006).
57. G. Ahlgren and L. Merino, *Arc. Hydrobiol.*, **121**, 295 (1991).
58. K.E. Harrison, *J. Shellfish Res.*, **9**, 1 (1990).
59. D.C. Müler-Navarra, *Limnol. Oceanog.*, **40**, 1209 (1995).
60. L.R. D'Abramo and S.S. Sheen, *Aquaculture*, **115**, 63 (1993).
61. X. Xu, W. Ji, J.D. Castell and R. O'Dor, *Aquaculture*, **118**, 277 (1993).
62. H.A. Vanderploeg, J.R. Liebig and A.A. Gluck, *J. Great Lakes Res.*, **22**, 36 (1996).
63. N.H. Norsker and J.G. Støttrup, *Aquaculture*, **125**, 155 (1994).
64. T. Watanabe, M. Ohta, C. Kitajima and S. Fujita, *Bull. Japan. Soc. Sci. Fish.*, **48**, 1775 (1982).
65. C. Desvilettes, G. Bourdier, J.C. Breton and P. Combrouze, *J. Plankton Res.*, **16**, 643 (1994).
66. M.C. Ederington, G.B. McManus and H.R. Harvey, *Limnol. Oceanogr.*, **40**, 860 (1995).
67. J.C. Léveillé, C. Amblard and G. Bourdier, *J. Plankton Res.*, **19**, 469 (1997).
68. T. Kaneda, *Microbiol. Rev.*, **55**, 288 (1991).
69. D.W. Stanley-Samuelson, *Comp. Biochem. Physiol. B*, **77**, 443 (1984).
70. M.W. Provost and N. Branch, *Fla. Entomol.*, **42**, 49 (1959).
71. A. Ali, *J. Am. Mosq. Cont. Assoc.*, **6**, 84 (1990).
72. A. Ali, R.J. Lobinske and P.K. Chaudhuri, *J. Am. Mosq. Cont. Assoc.*, **12**, 106 (1996).
73. A. Ali, J. Frouz and R.J. Lobinske, *Hydrobiologia*, **470**, 181 (2002).
74. A.P.M. Mourao and A.R. Panizzi, *Anais. Da. Sociedade. Entomol. Do. Brasil.*, **29**, 219 (2000).