Supplemental Information File

Direct and indirect responses of a freshwater food web to a potent synthetic estrogen

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Effects on Algae, Bacteria and Zooplankton

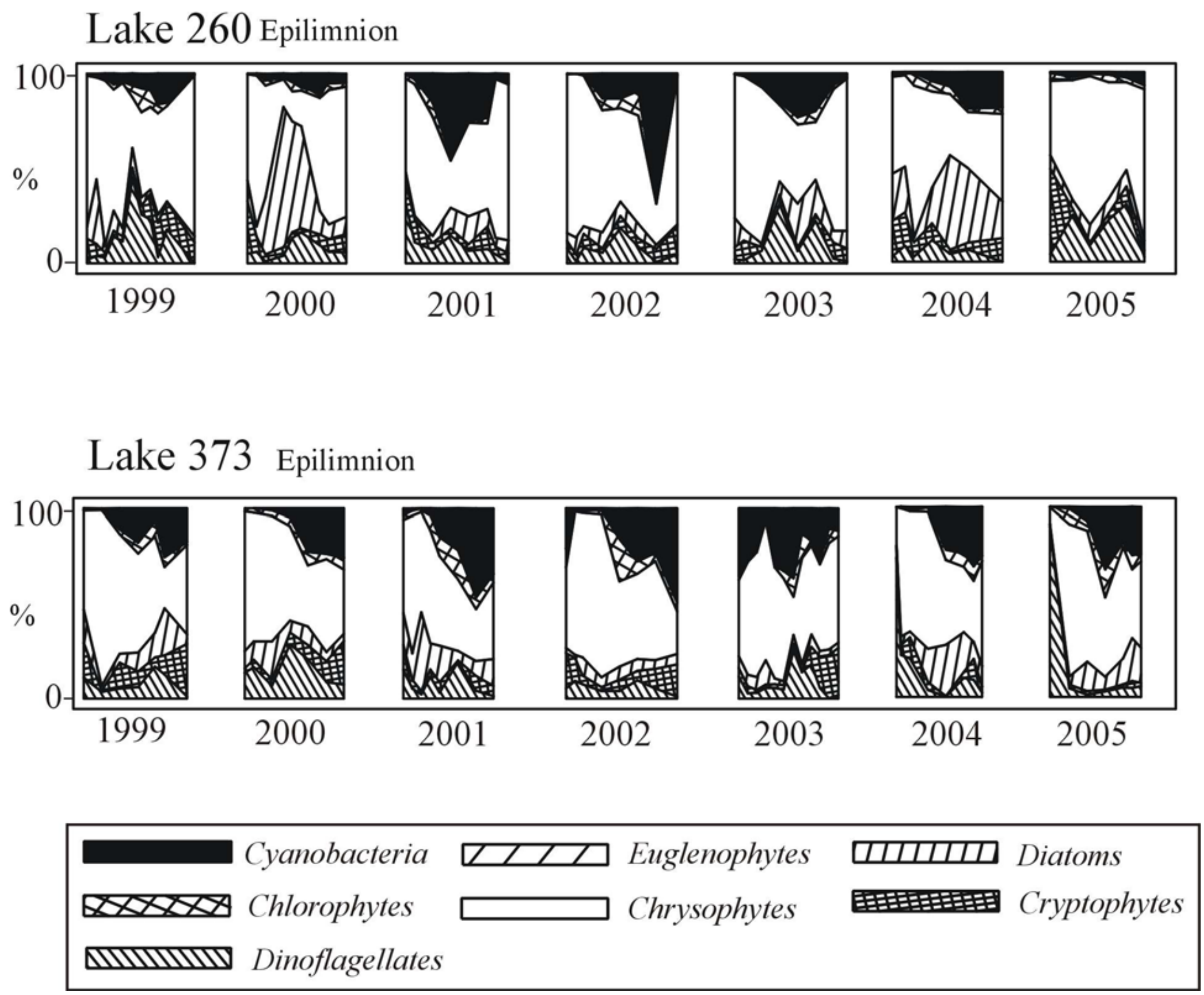

Figure S1: Composition of the epilimnetic phytoplankton community of Lake 260 (panel A) and Lake 373 (reference; panel B) before (1999, 2000), during (2001 – 2003) and after (2004 – 2005) EE2 additions to Lake 260.

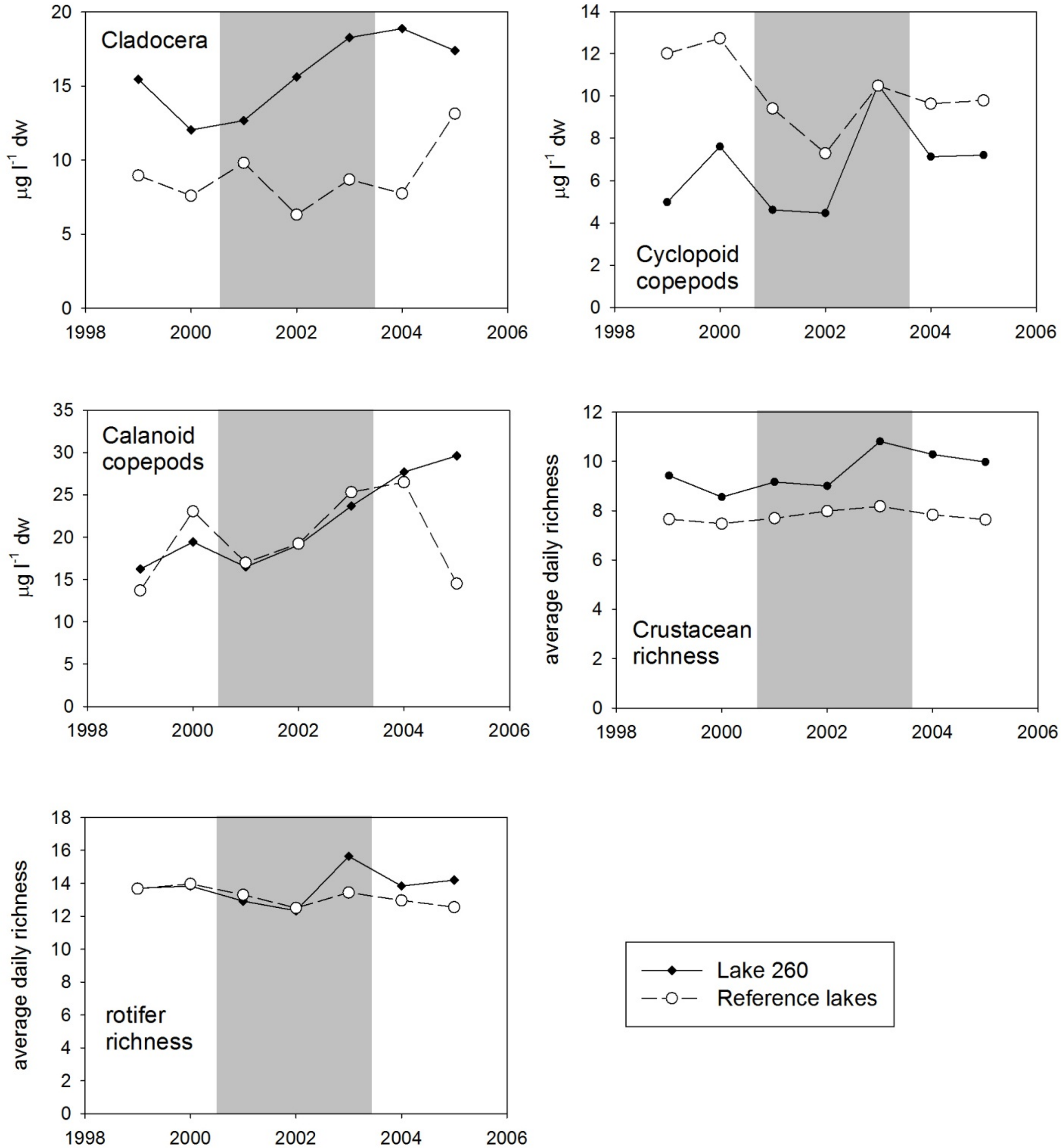

Figure S2: Annual mean biomass and species richness for zooplankton groups in Lake 260 and unimpacted reference lakes. Lakes 224, 239, 373 and 442 were used for Crustacea (including Cladocera, Calanoids, Cyclopoids) and Lakes 224 and 239 for rotifers.

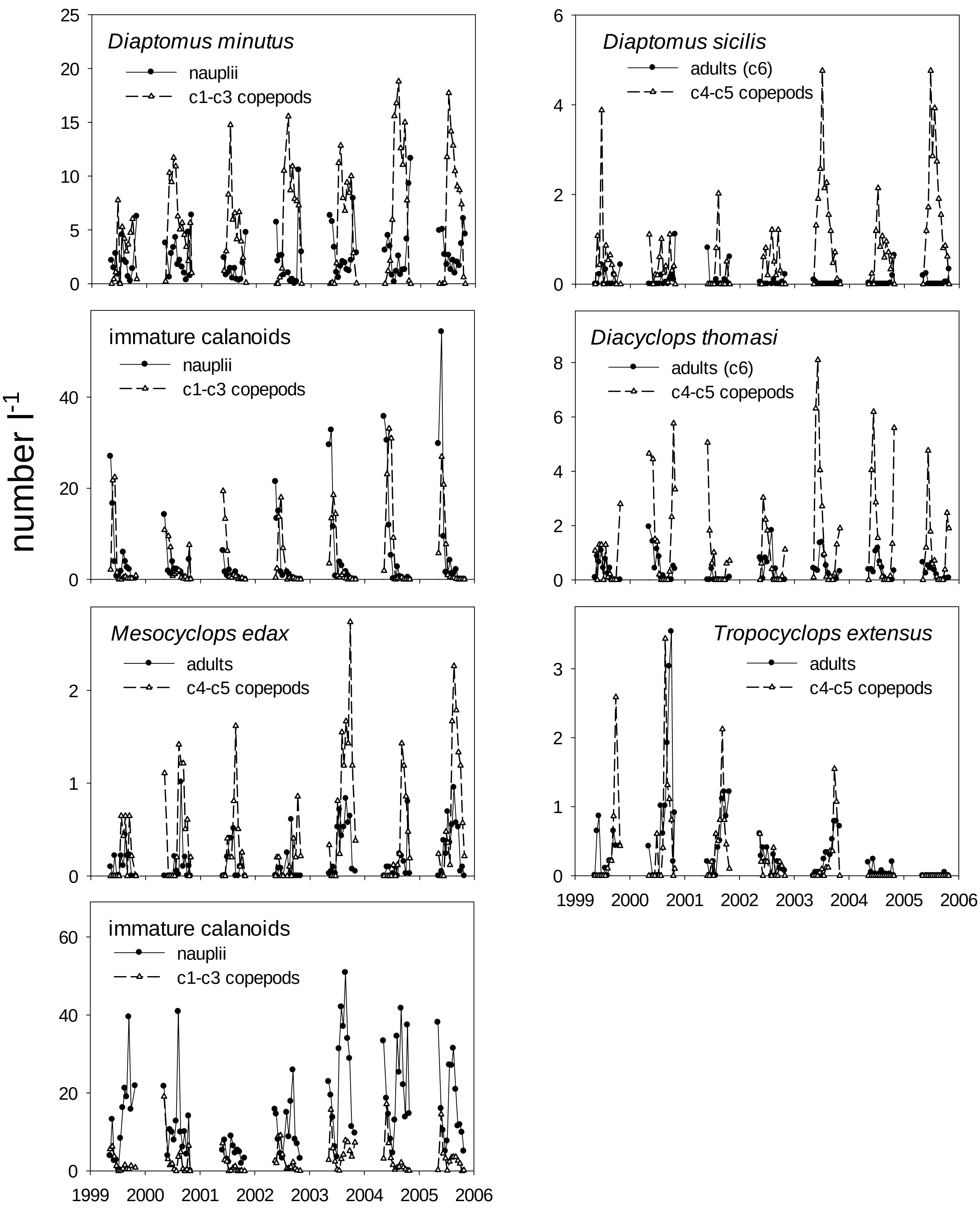

Figure S3a: Densities of common crustacean copepod taxa in Lake 260.

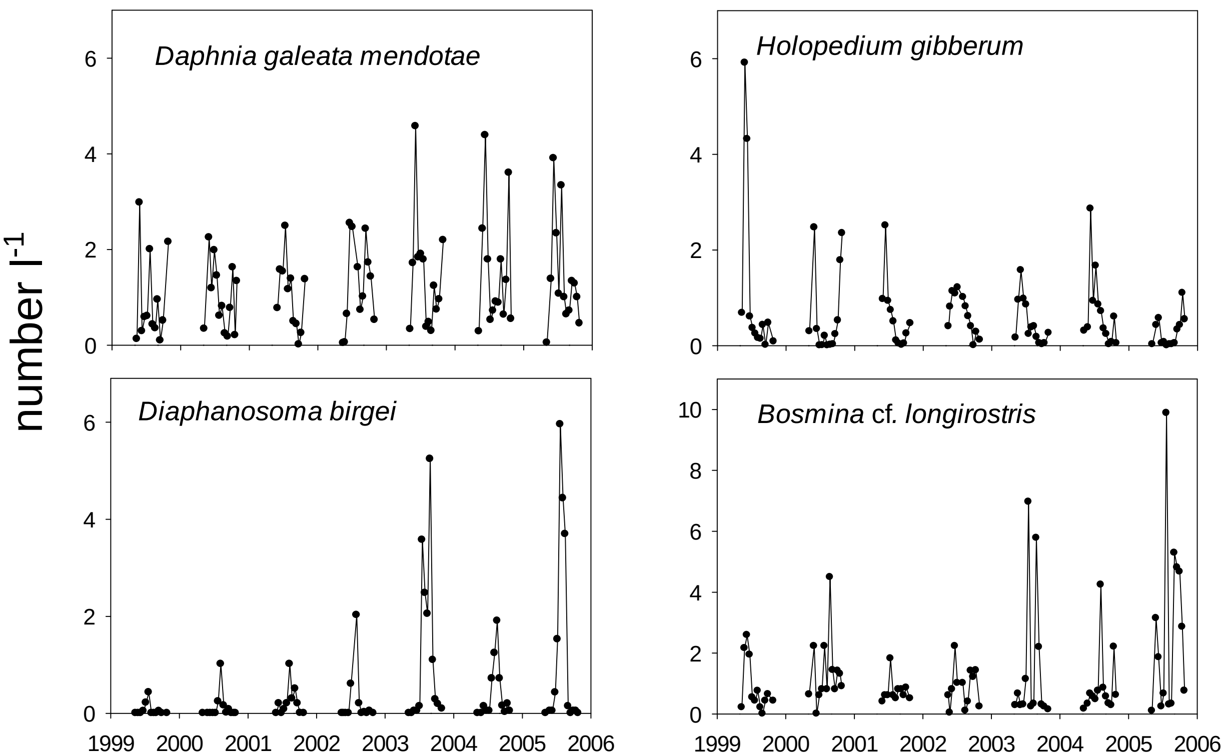

Figure S3b: Densities of common crustacean Cladocera in Lake 260.

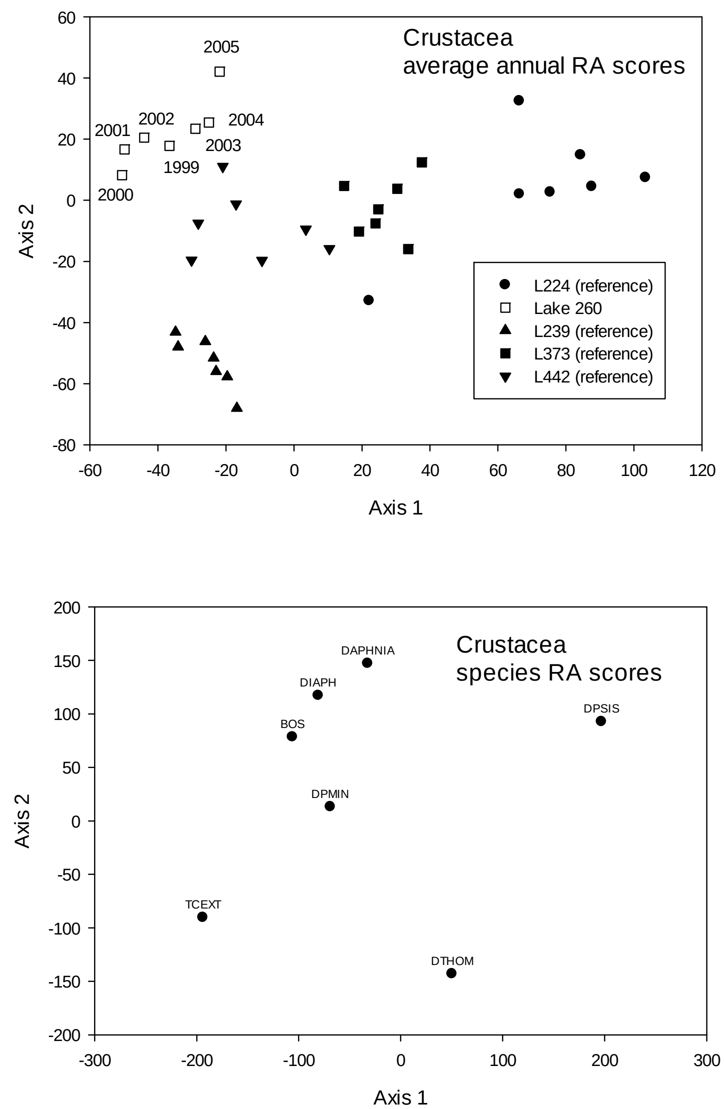

Figure S4: (a) Average Reciprocal Averaging (RA) scores for lake years in experimental Lake 260 and four unimpacted reference lakes. Labels denote different lake years in Lake 260; (b) RA loadings on axes 1 and 2 for common zooplankton crustacean taxa (abbreviations: BOS – *Bosmina* spp., DAPHNIA – *Daphnia* spp., DIAPH – *Diaphanasoma birgei*, DPMIN – *Diaptomus minutus*, DPSIS – *D. sicilis,* DTHOM – *Diacyclops thomasi*, TCEXT – *Tropocyclops extensus*).

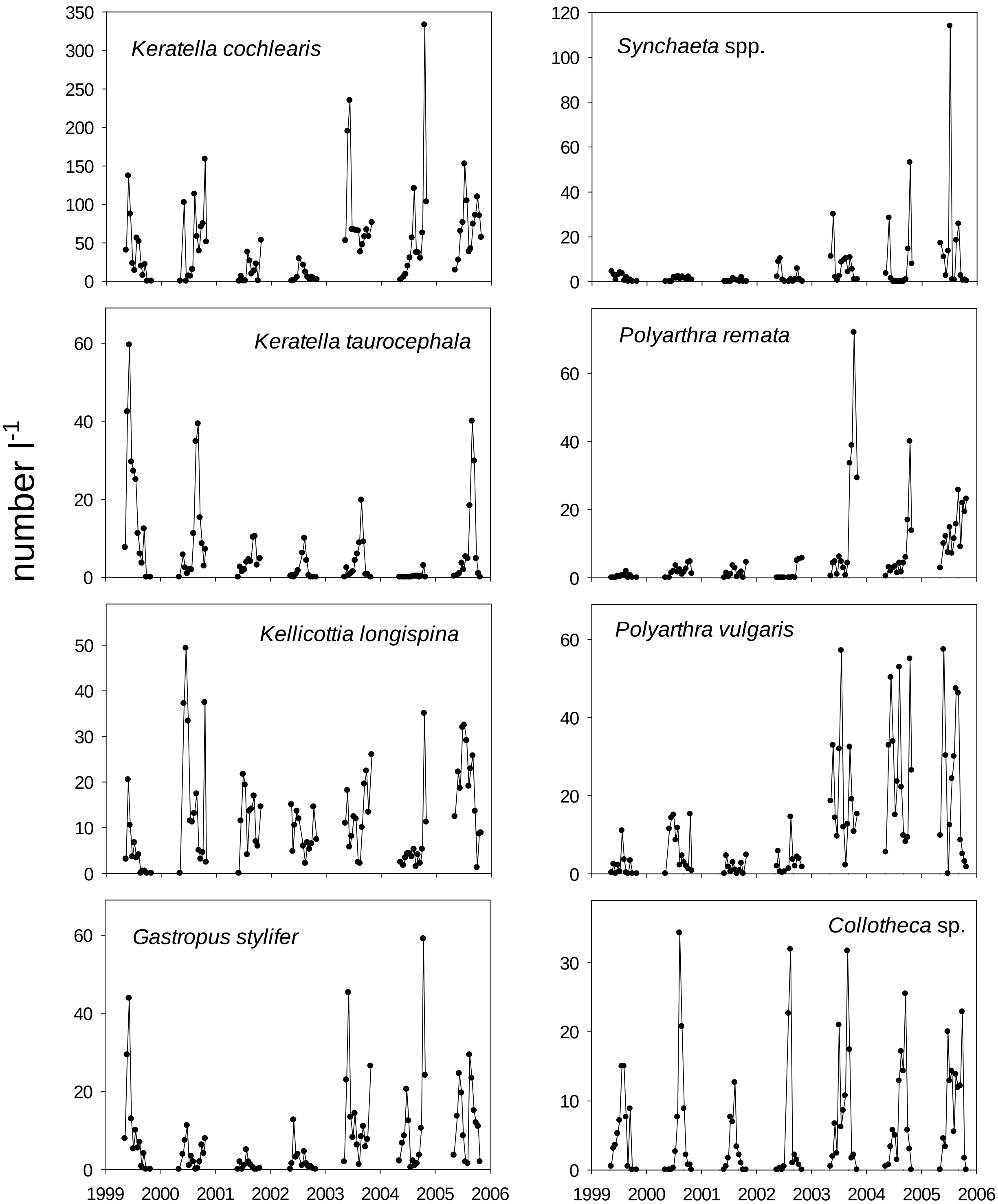

Figure S5: Densities of common rotifer taxa in Lake 260.

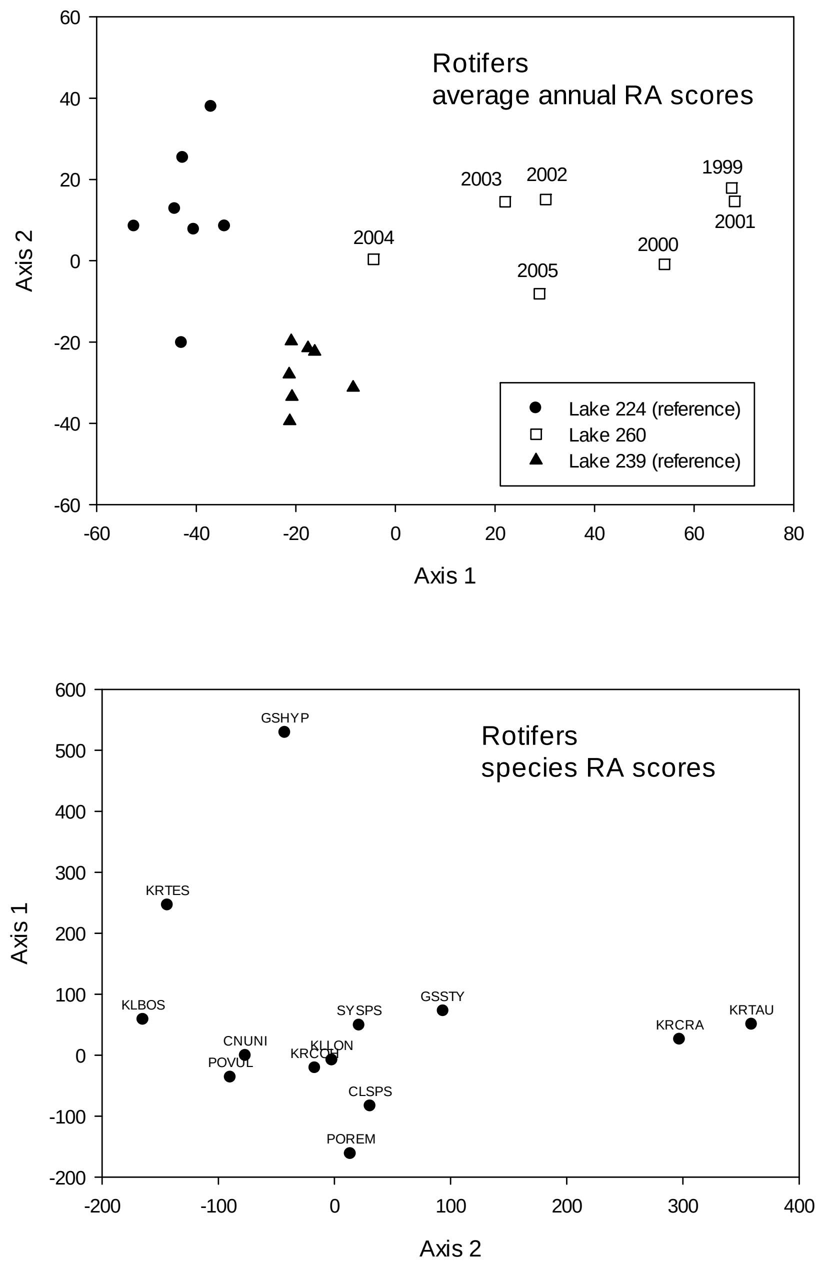

Figure S6: (a) Average Reciprocal Averaging (RA) scores for lake years in experimental Lake 260 and two unimpacted reference lakes. Labels denote different lake years in Lake 260; (b) RA loadings on axes 1 and 2 for common rotifer taxa (abbreviations: CLSPS – Collotheca spp., CNUNI – *Conochilus unicornis*, GSHYP – *Gastropus hyptopus*, GSSTY – *G. stylifer*, KLBOS – *Kellicottia bostoniensis*, KLLON – *K. longispina*, KRCOH – *Keratella cochlearis*, KRCRA – *K. crassa*, KRTAU – *K. taurocephala*, KRTES – *K. testudo*, POREM - *Polyarthra remata,* POVUL – *P. vulgaris,* SYSPS - *Synchaeta* sp.).

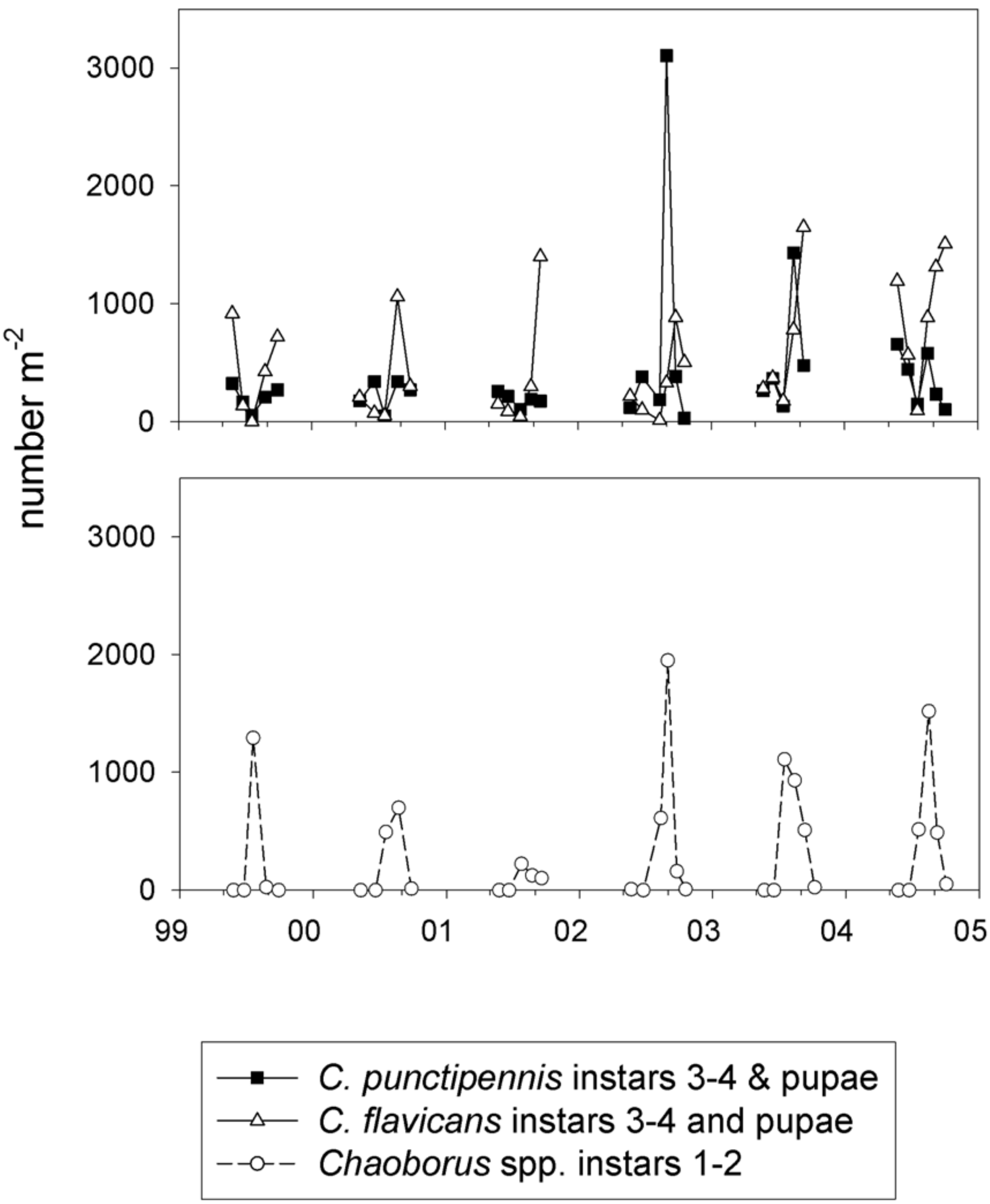

Figure S7: Changes in densities of different instars of *Chaoborus* taxa in Lake 260.

__Effects on Larval and Adult Littoral Invertebrates__

Emergence Traps

In addition to the increased total invertebrate emergence observed in Lake 260 in 2002 (see Figures 1), we also observed a change in the timing of the emergence of the males and females. Male chironomids typically emerge prior to the females and this phenomenon is called protandry. In 2001 in Lake 260, the males and females emerged at the same time suggesting some impacts of EE2 on this behaviour (Figure S9).

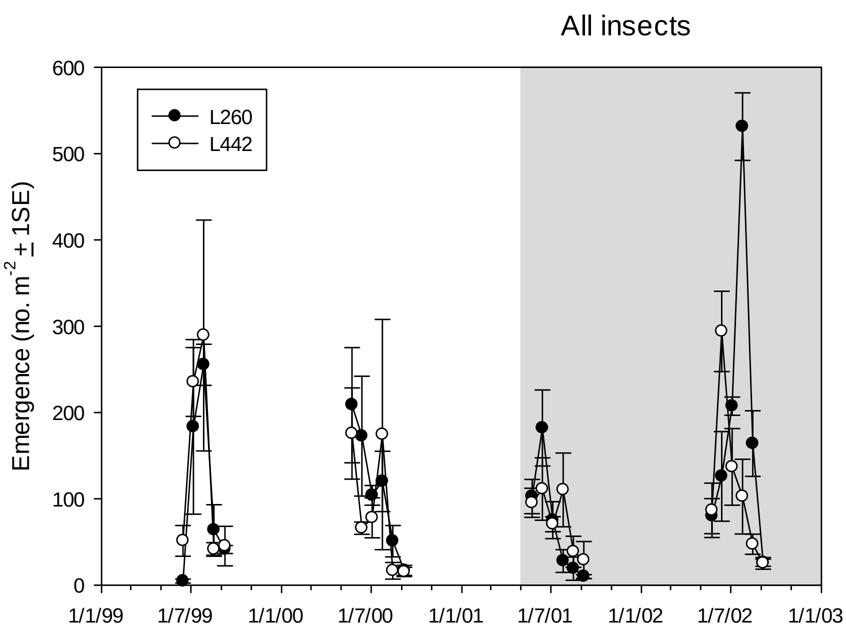

Figure S8a: Weekly emergence (mean ± SE; n=3/date) of all insects emerging from Lake 260 (solid symbols) and reference Lake 442 (open symbols) before (1999-2000) and during (2001-2002) EE2 additions to Lake 260.

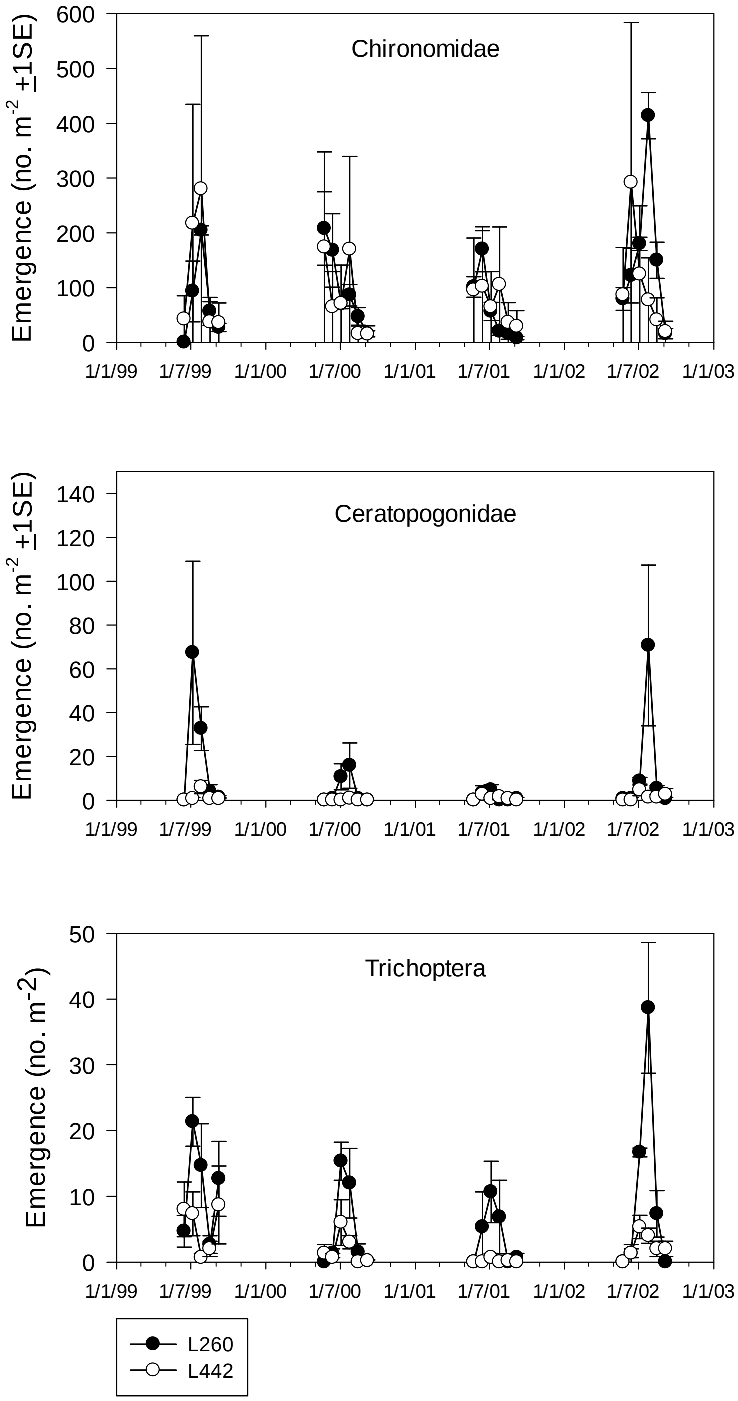

Figure S8b: Weekly emergence (mean ± SE; n=3/date) of chironomids, ceratopogonids, and trichopterans from Lake 260 (solid symbols) and reference Lake 442 (open symbols) before (1999-2000) and during (2001-2002) EE2 additions to Lake 260.

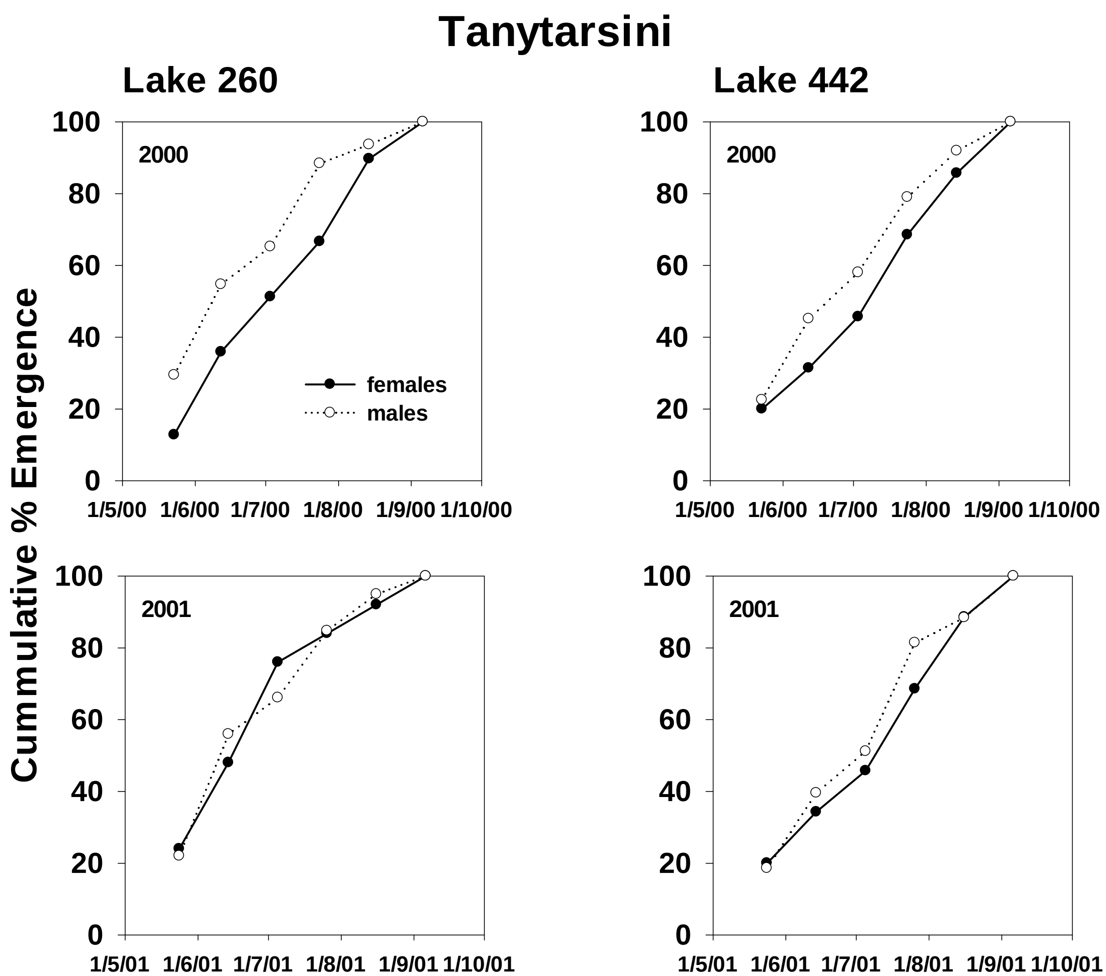

Figure S9: Cumulative emergence of male and female chironomids (Family Chironomidae, Tribe Tanytarsini) in Lake 260 before (2000) and during (2001) EE2 additions and in the reference Lake 442 over the same time period.

Artificial Substrates

Rock baskets provide standardized samples from rocky lake substrates that are not easily sampled by more conventional means such as dredges. Three sites representing the three most common hard-bottom habitat types in L260 and L442 were selected in both lakes. Baskets (0.01 m^{-3}) containing local cobble were placed in 70 cm depth of water in the fall and left to colonize over the winter and following summer. The three habitat types were 1) large rocks, 2) large rocks and woody debris, and 3) cobble. Nine rock baskets from each lake (3 per site) were collected and processed each of three times during the summers of 2000 and 2001. Baskets were collected and rocks were washed individually with a 250 µm mesh. Invertebrates were preserved initially with 10% formalin and were later transferred to 70% ethanol. Animals were identified to family where possible using Merritt and Cummins (1996) and Thorp and Covich (1991).

A BACI analysis was conducted to determine if EE2 have a significant effect on the abundance of invertebrates on the artificial substrates. Invertebrate groups chosen for this analysis were those taxa collected in the emergence traps and those groups for which some studies on estrogen exposures have been reported in the literature. The values from the 3 baskets collected at each habitat were averaged for each sampling date. The habitat types were paired for each sampling date and the difference in the number of invertebrates per basket in Lakes 260 and 442 sites was calculated. The values for the differences were then analysed using a 2-way ANOVA with habitat and year as fixed factors. Habitat was a fixed factor because the three areas sampled had been chosen specifically as representatives of the 3 most common littoral habitats in the two study lakes. A significant effect of EE2 was indicated by a significant Year effect.

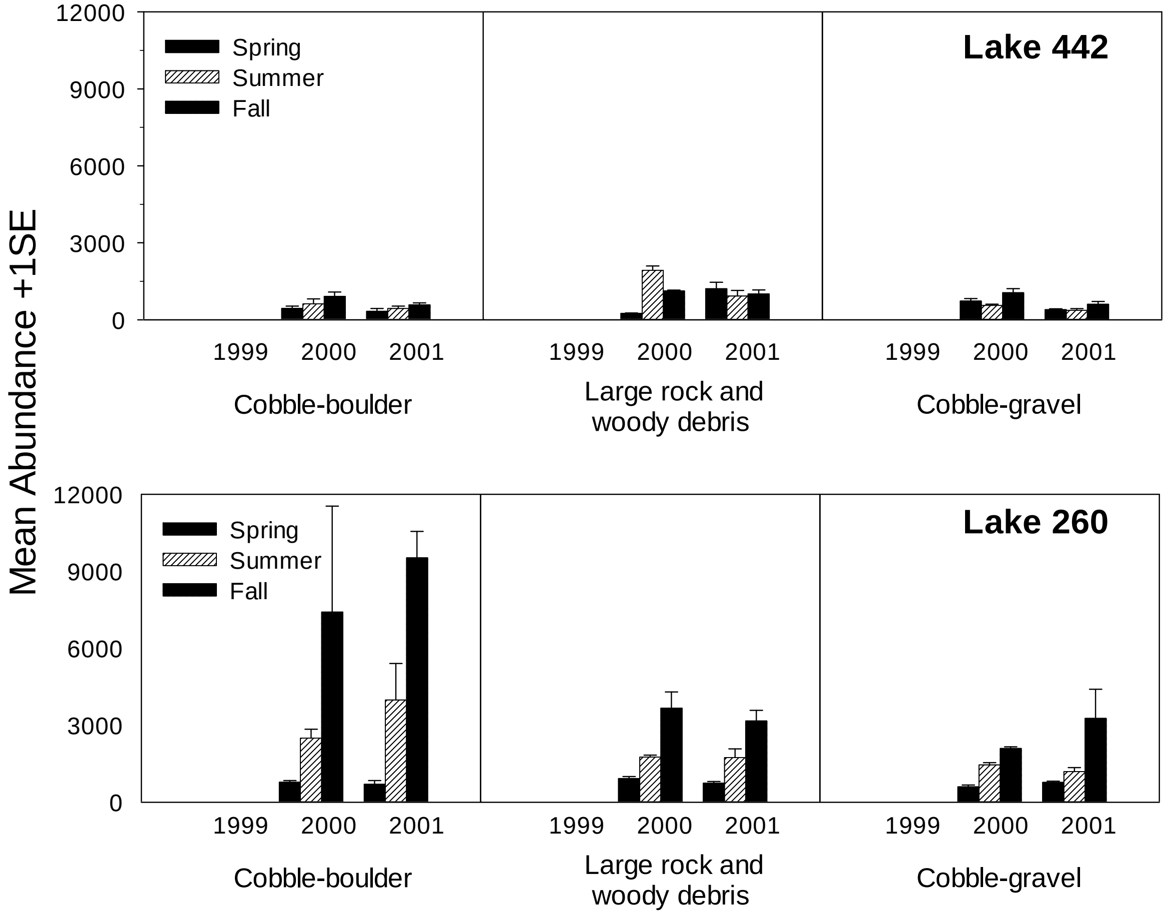

Figure S10: Mean (total abundance of larval invertebrates collected on rock baskets placed in three different substrates in the reference (Lake 442) and experimental lake (Lake 260) in the year before (2000) and year during (2001) EE2 additions to Lake 260.

Table S1: Results of two-way BACI ANOVAs comparing differences between littoral macroinvertebrates collected on artificial substrates from three different habitats in spring, summer and fall from Lake 260 and from unimpacted reference Lake 442 before (2000) and during (2001) EE2 additions to Lake 260. Significant differences * at α/m=0.007.

Group	**F**	**p**
Chironomidae	2.29	0.15
Ceratopogonidae	0.11	0.95
Sphaeridae (Bivalvia)	0.03	0.86
Gastropoda	0.28	0.61
Talitridae	3.83	0.071
Trichoptera	0.55	0.47
Total invertebrates	11.46	0.004*

Total df=17, habitat df = 2, year df = 1, Error df = 14

Effects on Fish Populations and Community

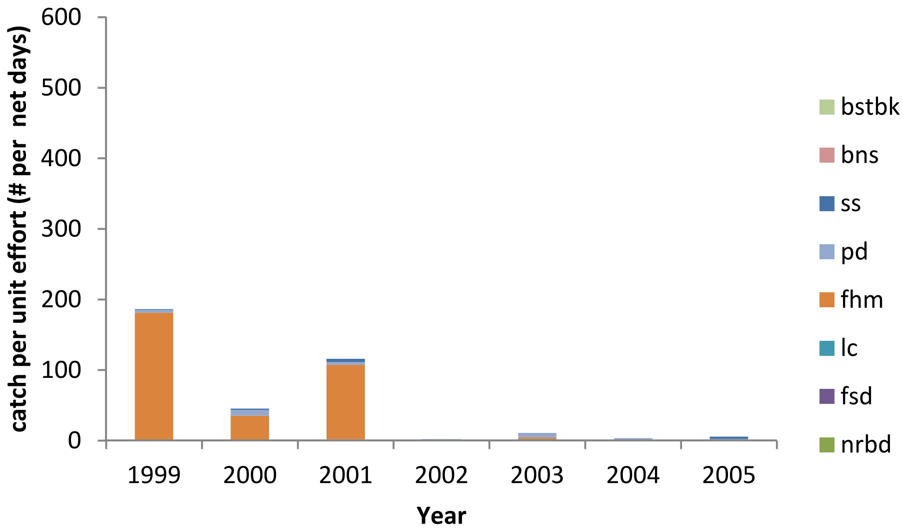

Figure S11: Small fish relative abundance in Lake 260 based on fall catch-per-unit-effort. Species as follows: nrbd = northern redbelly dace, fsd = finescale dace, lc = lake chub, fhm = fathead minnow, pd = pearl dace, ss = slimy sculpin, bns = blacknose shiner, bstbk = brook stickleback.

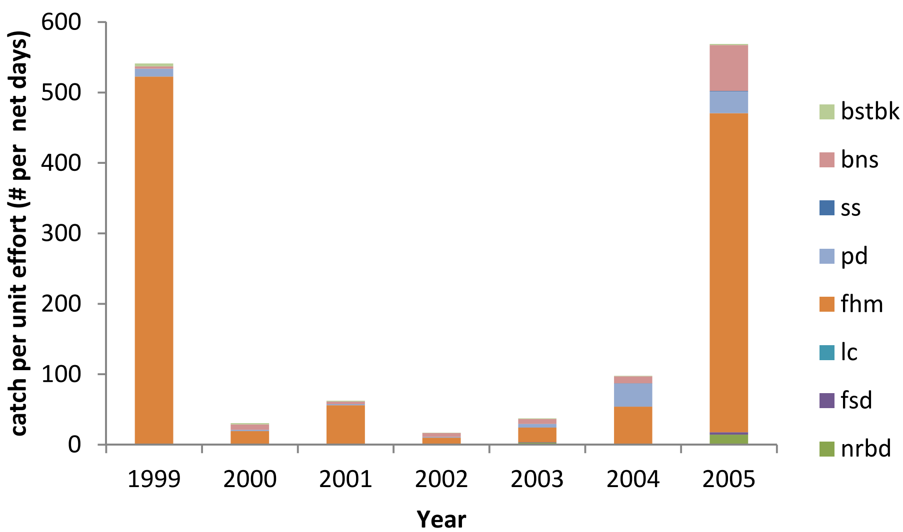

Figure S12: Small fish relative abundance in Lake 442 based on fall catch-per-unit-effort. Species abbreviations as in Figure S11.

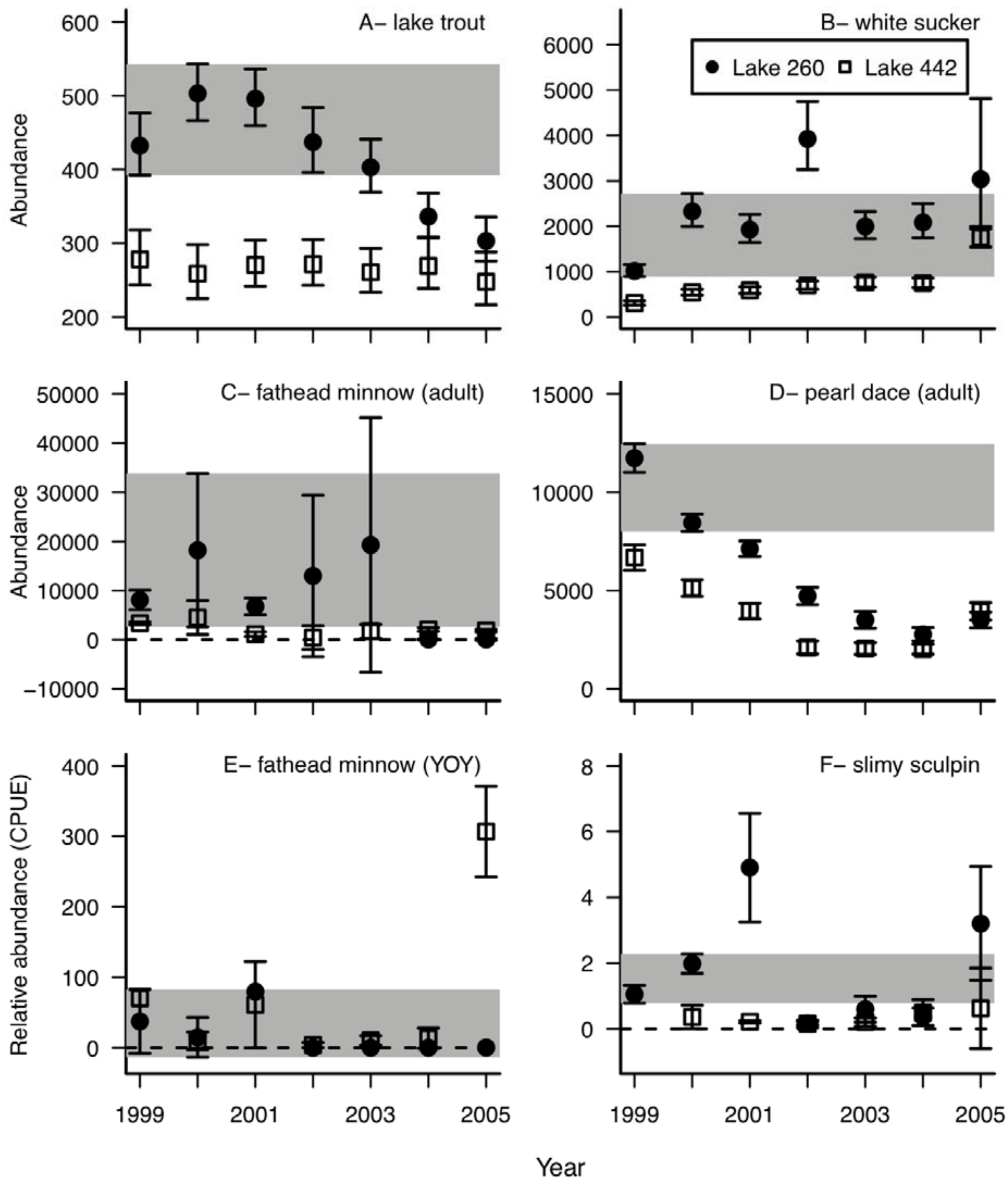

Figure S13: Abundance of fishes in Lakes 442 and 260. Error bars are 95% confidence intervals; points missing error bars lack sufficient replication to permit determination of error. Grey shading represents upper and lower bounds of 95% confidence intervals for abundance estimates prior to EE2 additions in Lake 260.

Table S2: Results of Tukey's tests (*p*-values) evaluating differences in fish condition among time periods in the experiment: Pre-addition (1999-2000), during EE2 addition (2001-2003), and post-addition (2004-05). Dash indicates no significant differences (p >0.05) between time periods.

Lake	Species	Sex	EE2 vs. Pre	EE2 vs. Post	Pre vs. Post
260	Lake trout	Female	–	–	–
		Male	<0.0001	<0.0001	–
	White sucker	Female	<0.0001	<0.0001	–
		Male	<0.001	<0.001	–
224	Lake trout	Female	–	< 0.0001	0.002
		Male	–	0.007	<0.001
	White sucker	Female	–	0.0008	–
		Male	–	–	–
373	Lake trout	Female	–	–	–
		Male	–	–	–
	White sucker	Female	–	–	–
		Male	–	–	–
442	Lake trout	Female	–	–	–
		Male	–	–	–
	White sucker	Female	0.0003	–	–
		Male	<0.0001	–	<0.0001

References

Merritt RW, Cummins KW. 1996 *An Introduction to the Aquatic Insects of North America*. Kendall Hunt.

Thorp JH, Covich AP. 1991 *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc. San Diego.