Experimental Infection of Bonytail Chub (*Gila elegans*) with the Cestode *Bothriocephalus acheilognathi*

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Two year (99-00) seasonal parasitological survey

11 species: 1435 fish

Native	n	Length (mm)
Bluehead sucker	148	35-288
Flannelmouth sucker	73	36-492
Humpback chub	116	34-147
Speckled dace	630	29-115
Non-native		
Channel catfish	54	48 - 770
Yellow bullhead	12	80 - 252
Common carp	63	30 - 600
Fathead minnow	193	31 - 99
Plains killifish	113	28 - 81
Rainbow trout	22	252 - 441
Red Shiner	11	50 - 88

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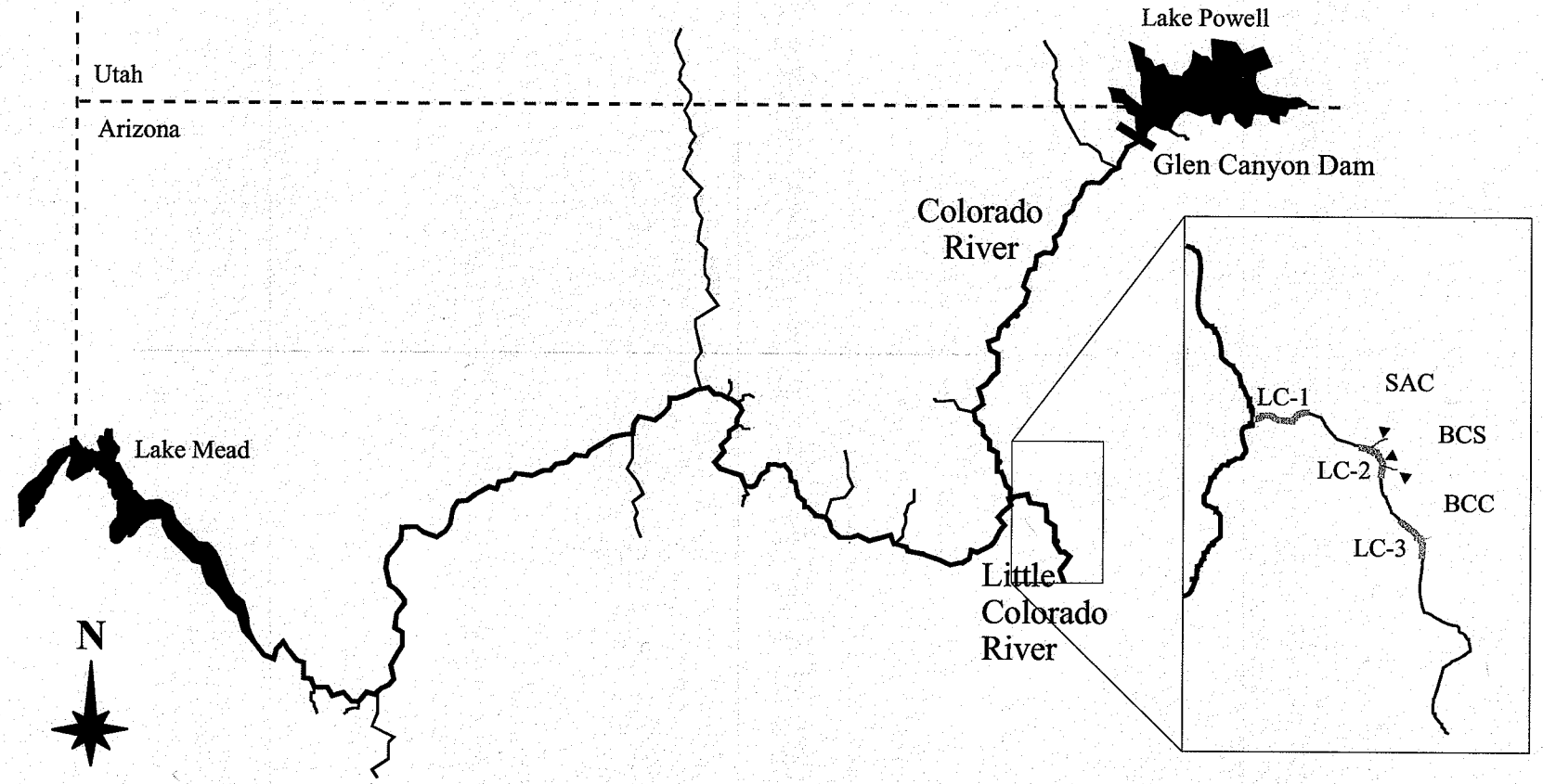
Sampling Sites in LCR



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Parasite Species	BHS	FMS	HBC	SPD	CCF	CRP	FHM	PKF	RBT	RSH	YBH
Myxozoa											
Henneguya exilis					X						
Myxosporea gen. Sp.								X	X		
Monogenea											
Ligictaluridus sp.					1.13						
Dactylogyrus sp.1						0.27					
Dactylogyrus sp.2				0.04			0.24				
Cestoda											
Bothriocephalus acheilognathi	0.05	0.07	18.4	1.97	0.03	35	0.84	1.26	2.95	1.2	0.08
Corallobothrium fimbriatum					1.96						
Megathylacoides giganteum					1.59						
Trematoda											
Ornithodiplostomum sp.	0.06	0.01	7.7	1.53	0.02		0.24				
Posthodiplostomum sp.			0.01	0.01			0.02				
Nematoda											
Rhabdochona sp.			0.11	0.37				0.01			
Truttaedacnitis truttae									24.8		
Eustrongylides sp.					0.24						
Contracaecum sp.					0.18					0.09	0.08
Hirudinea											
Myzobdella lugubris					0.02						
Copepoda											
Ergasilus sp.					0.02				0.04		
Lernaea cyprinacea			0.07	0.01							
Acari				0.02				0.02			

Summary

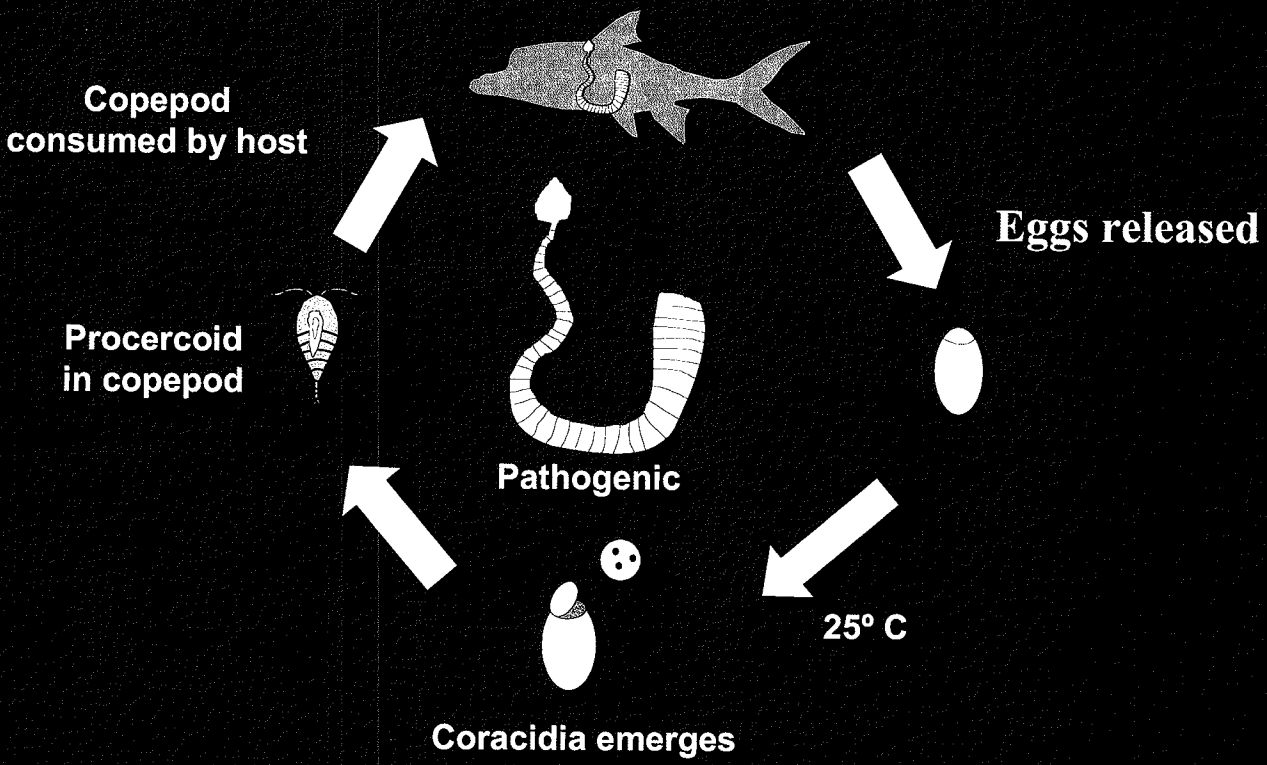
- Numbers of *B. acheilognathi* increased with length of HBC.
- *B. acheilognathi* was found in all species of fish but was most abundant in cyprinids.
- While HBC made up only 8 % of the sample, they harbored over 54% of the *B. acheilognathi*.
- No signs of clinical disease were noted, however very small HBC were not available for sampling.

Bothriocephalus acheilognathi

- **History**
  - **First identified in North America in 1975**
  - **Presumably introduced with grass carp**
  - **Discovered in Colorado River in 1994**

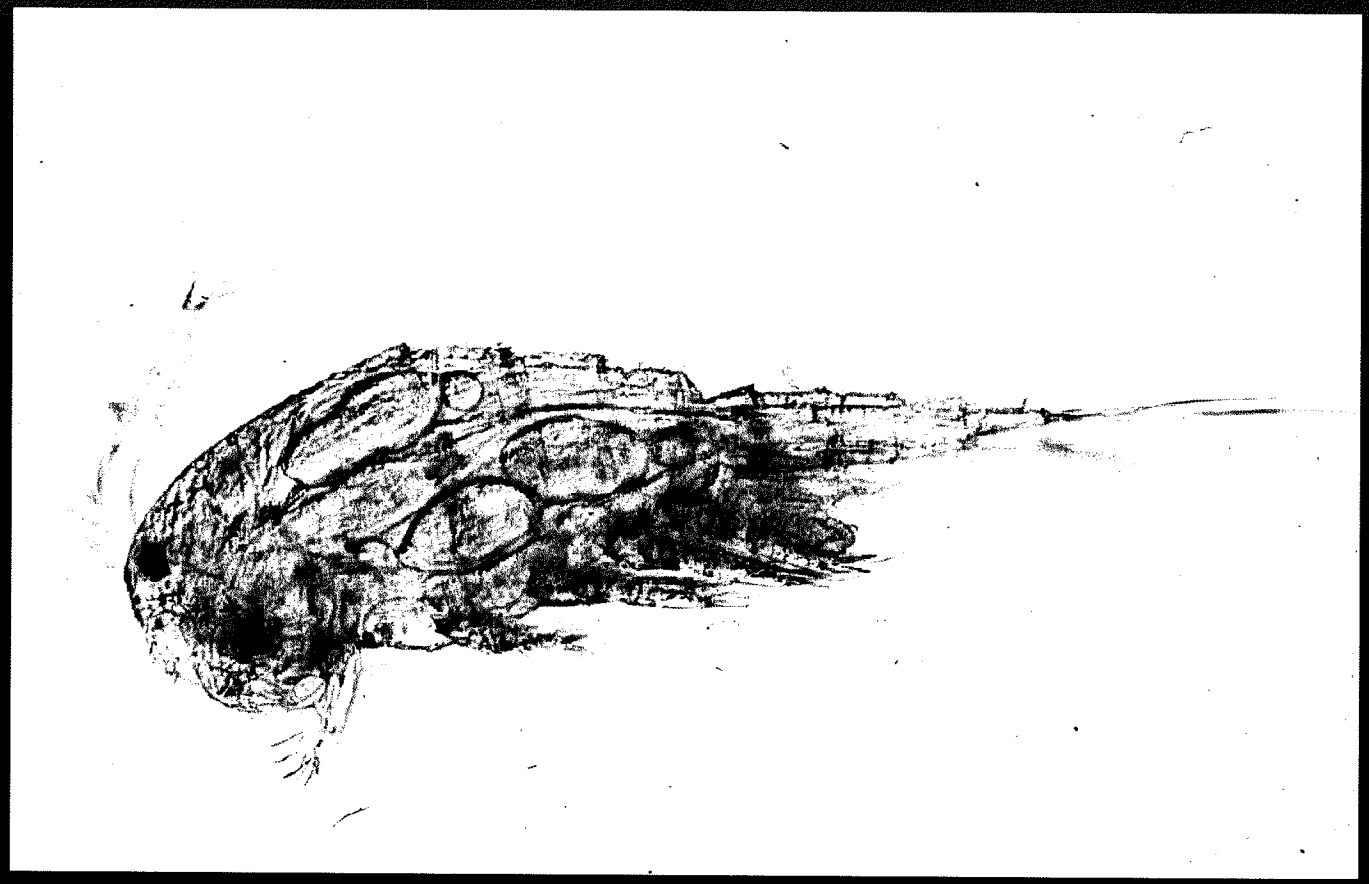
- **Effects on fish**
  - **Extensive mortality in hatchery-reared juvenile carp**
  - **Impaired growth suggested in roundtail chub (Brouder, 1999)**
  - **Pathogenic effects may include perforation of the GI tract, degeneration and necrosis at the point of attachment, and gut impaction in small fish**

Life Cycle of *Bothriocephalus acheilognathi*



Optimal hatching temp is 25-30C, optimal temp for development of worm in fish is 25C. (Average temp in LCR is 24C, mainstem Colo River is 12C.)

Procercoid intensity greater in *A. robustus* from LCR as compared to *A. robustus* isolate from Colorado River



Laboratory Study Objectives

1. Morbidity/Mortality
2. Growth rates: infected vs uninfected
3. Pathogenic effects
   -includes health assessment based on Goede and Barton (1990)
4. Effects of thermal stress
   -simulate infected fish moving from LCR (24C) into CO River (12C)

Experiment #1 Design

- Bonytail chub (*Gila elegans*) used as surrogate
  - Biology similar to HBC: Successfully hybridize
  - Demonstrated suitability as tapeworm host in pilot studies
- Sample size = 160 (80 treatment /80 control)
  - Mean fork length = 83.20 mm (±13.38)
- Each fish given unique fluorescent elastomer mark (tag)
- Fish euthanized 13 wk post exposure

Exposed once to infected copepods

23	20	14	23

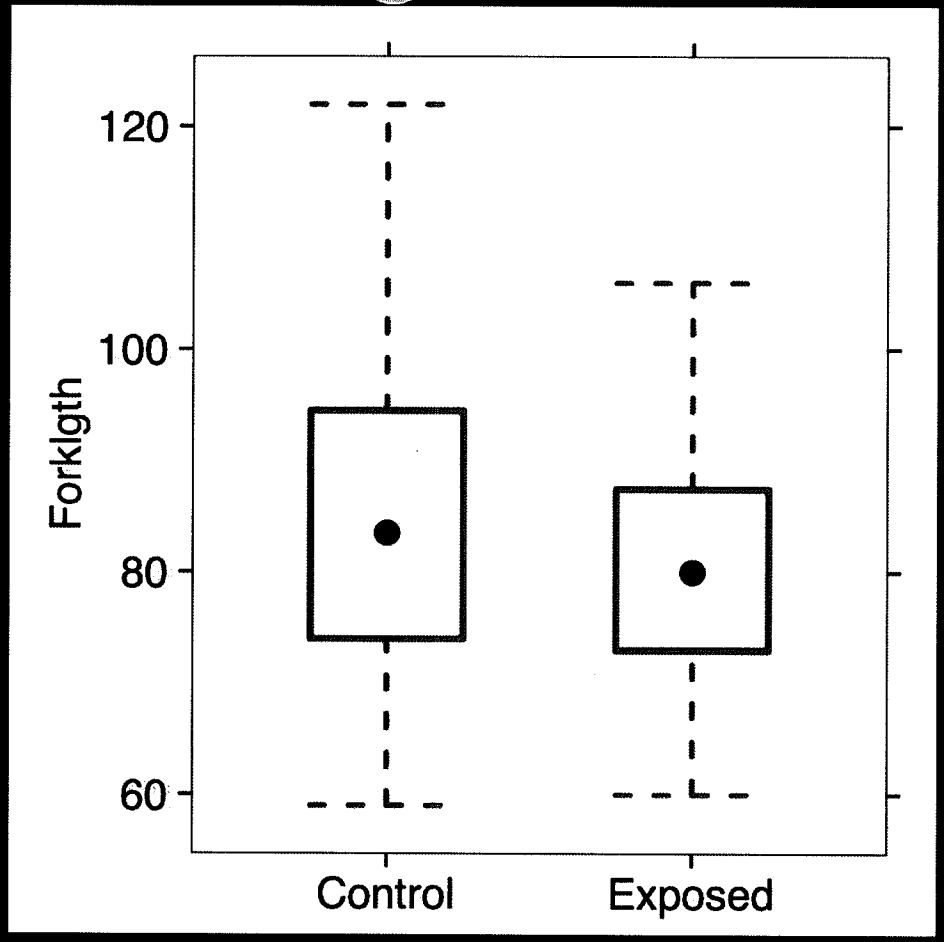
Unexposed

23	20	14	23

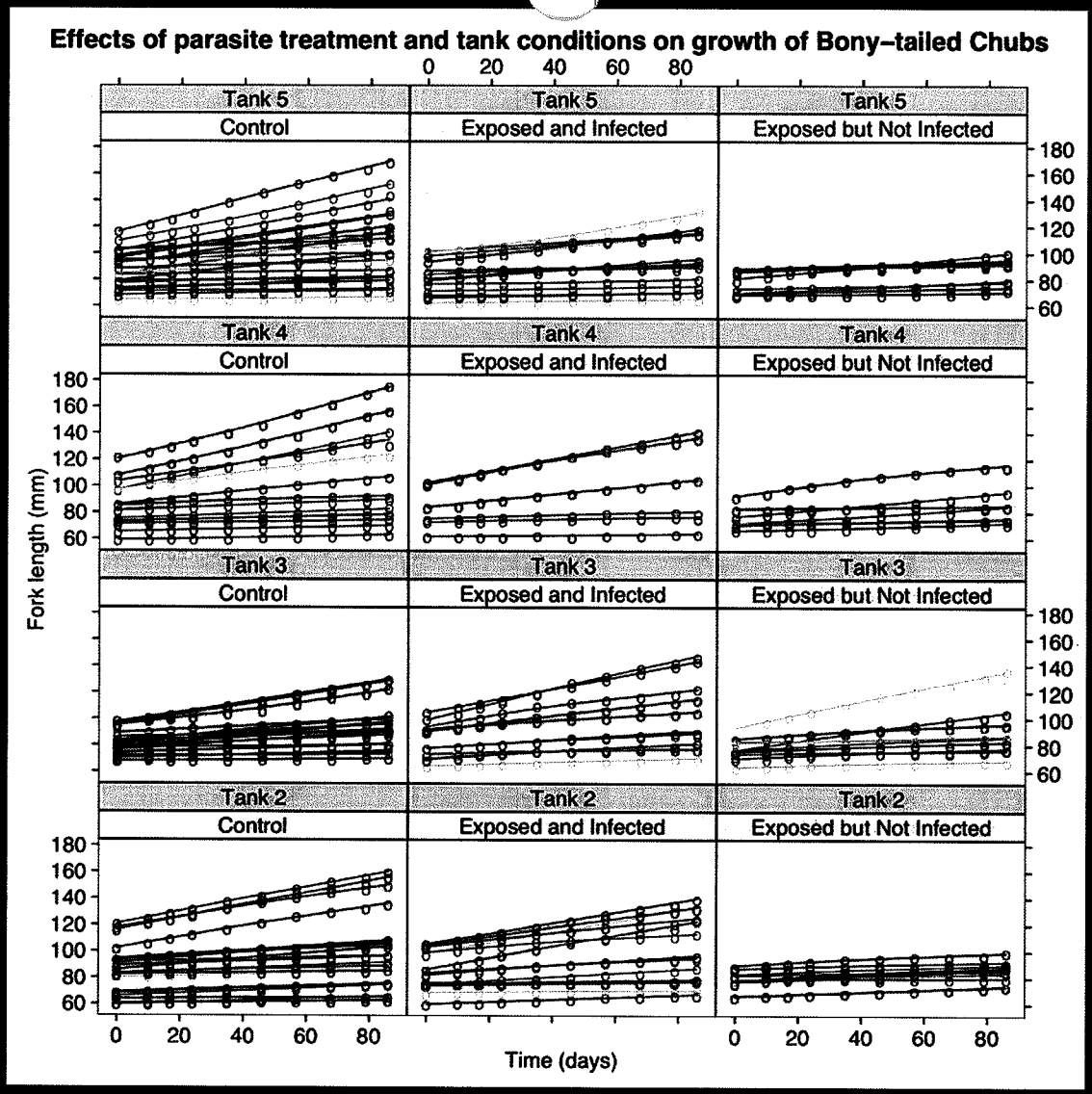
Tagged individual fish were measured & weighed every 7-10 days up to 86 days, fed 1% maintenance ration.

At necropsy, worm numbers, hematocrit, liver wt & condition determined

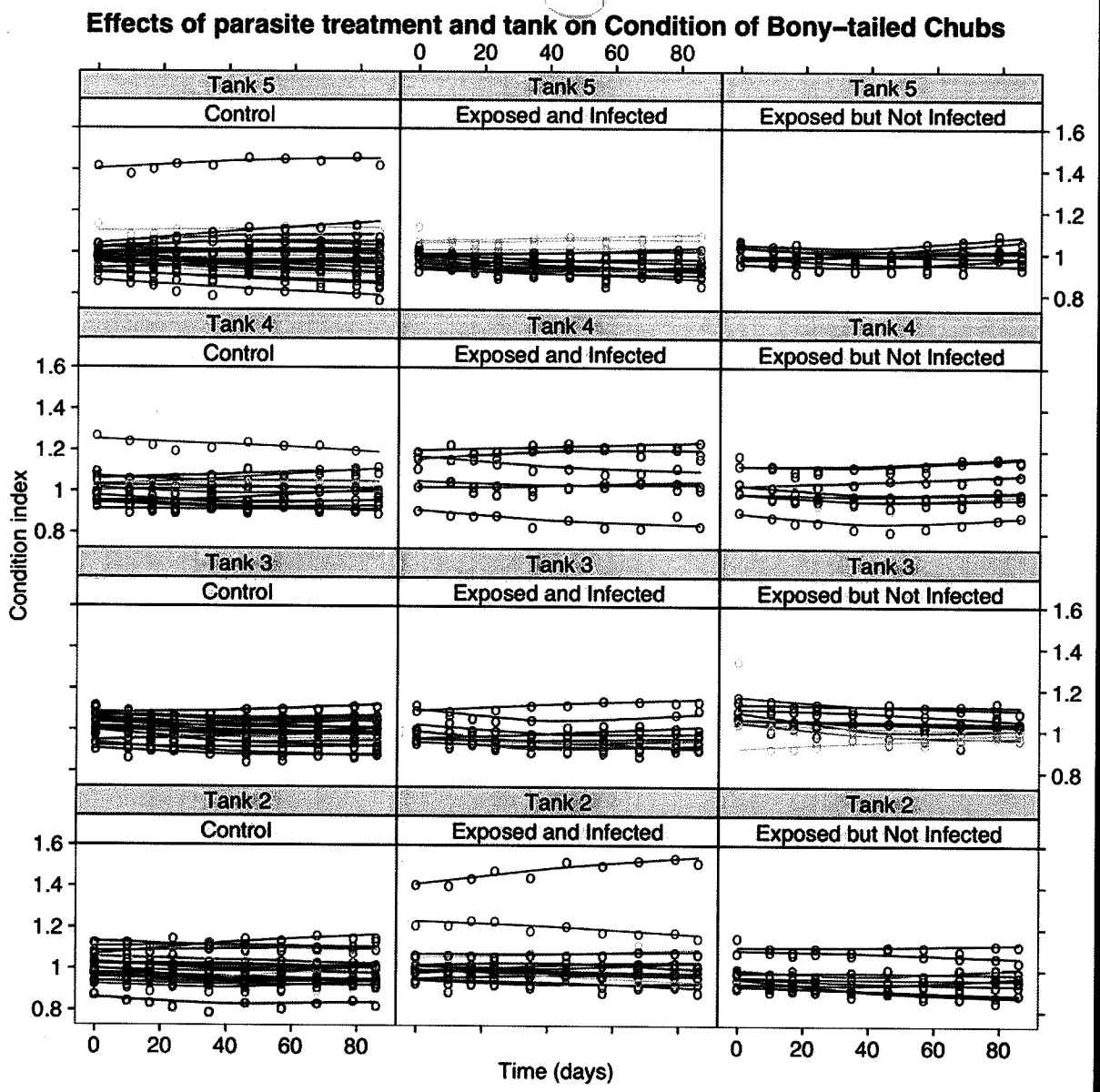
- 43 of 80 (54%) of exposed fish were infected
- Intensity of infection: 1 – 70 worms
- Mean intensity: 11.5 worms
- 67% of fish had <10 worms each



Even though fish were randomly allocated to treatment/control, there was a large variability in the initial size distribution of fish between exposed and control treatments. Fish from exposed tanks were significantly smaller in length at day 0 than control fish (F_1,158=3.94, P<0.05).



Growth rate of individual fish in control & exposed tanks. Fish that were exposed & infected are separated from fish that were exposed but not infected. Lines of different colors represent individual fish.



Change of body condition through time for individual fish

	Treatment	Tank	Density	Sex
Length (mm)	**0.02***	0.87	0.14	0.56
Log (weight)	0/05	0.96	0.21	0.42
B. Condition	0.64	0.13	0.60	**0.03***
Hematocrit	0.07	0.12	0.15	0.97
Liver Wt	0.12	0.84	0.24	0.27
L. Condition	0.30	0.16	0.19	0.29

P-values for linear models fitted to each of the response variables (left column) as a function of explanatory variables (column headers). Significant effects shown with bold asterisk.

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Summary of Experiment 1

- Difference in initial fish size distribution might have confounded the small effects of parasitism on growth.
- Growth rate of uninfected fish was significantly smaller than exposed infected or control fish.
- No significant effect on other variables except body condition which was lower in males

Experiment #2 Design

- **Bonytail chub (*Gila elegans*) used as surrogate**
- **Sample size = 120 (72 treatment /48control)**
  - Mean fork length = 22.28 mm (±2.14)
- **No individual tags due to small size; fed 1% maintenance ration.**
- **Fish euthanized 23 wk post exposure**

Experiment 2

Exposed to infected copepods

12 12 12 12 12 12

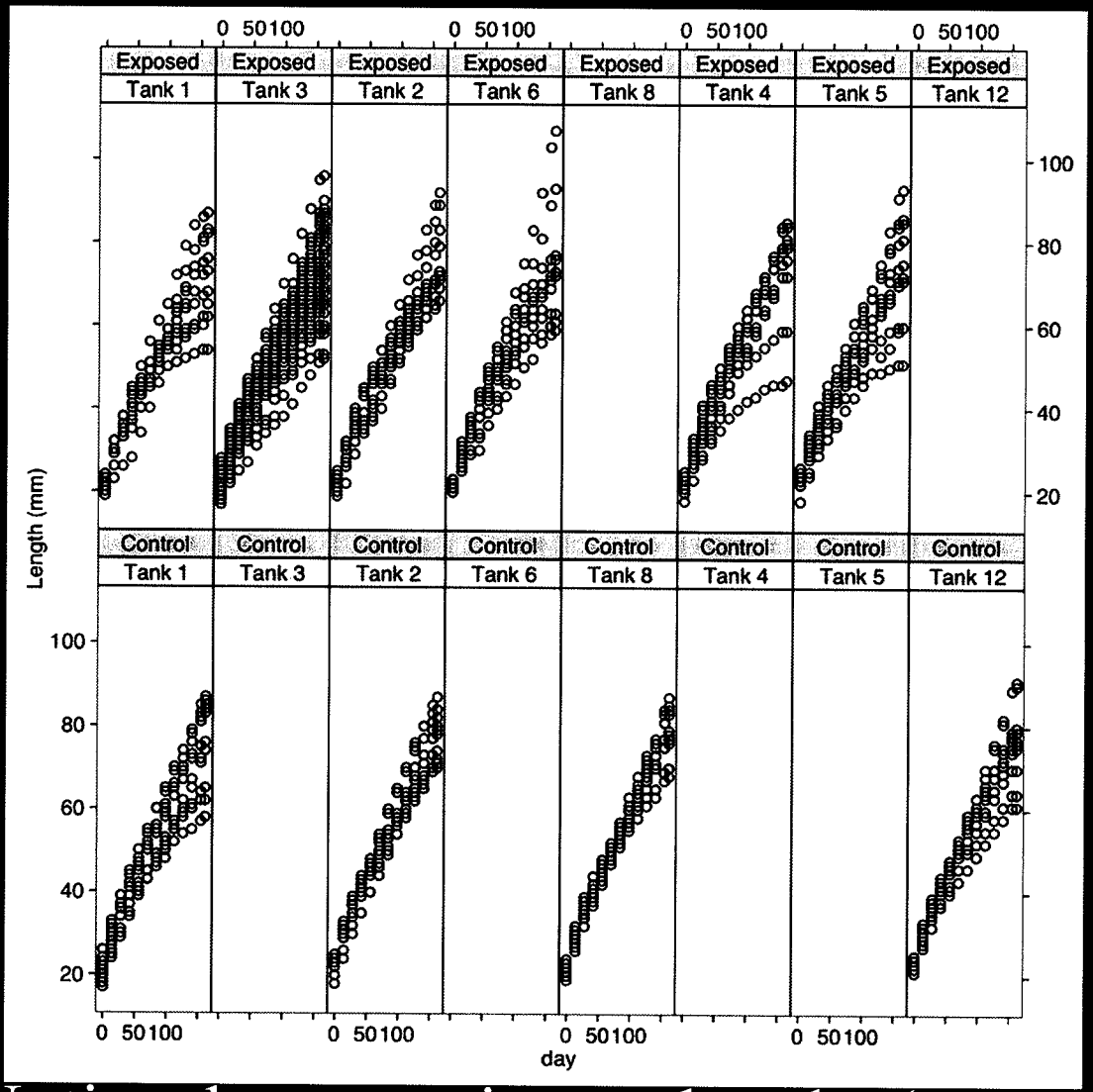
Not exposed

12 12 12 12

- Population in each tank was measured & weighed every 14 days until day 161 p.exp.
- At day 161,72 fish were chilled for 48 h (24 from control & 48 from exposed)
- At necropsy worm numbers, hematocrit, liver wt & condition were determined.

- 48% (44/92) infected (54% Exp 1)
- Intensity of infection: 1-151 worms (1-70 Exp 1)
- Mean intensity: 17.2 worms/infected fish (11.5 worms Exp 1)

Change in fish length as a function of time



Notice that experimental tanks (upper panels) are more variable than controls

	Treatment effect		Tank effect	
	Infected Only	Both	Infected Only	Both
Length	<0.01*	0.16	0.65	0.26
Log (weight)	<0.01*	0.12	0.63	0.96
B. Condition	0.17	0.45	0.42	0.01*
Hematocrit	<0.01*	0.28	0.29	0.37
Liver Weight	0.04*	0.24	0.74	0.15
Liver Condition	0.89	0.39	0.04*	<0.01*

Parasite exposure decreased length of infected fish by average of 6.8 mm, wt by 0.9 mg, hematocrit by 2.6 and liver wt by 0.01 mg. These effects were only obvious when uninfected fish were removed from exposed treatment and compared to the control.

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Effects of chilling fish for 48 h @ 12 C

- Effects on length, hematocrit, worm burden, liver weight, body condition and liver condition were not significant.

Summary of Experiment 2

- There was a large variability in size and condition of fish in exposed tanks, probably due to within-tank asymmetries originated by the exposure to parasites.
- Length, weight, hematocrit and liver weight were significantly smaller in exposed infected fish compared to control uninfected fish.
- Chilling for 48 h does not seem to affect any of the variables.

Points to Ponder

- Keep in mind that this was a one time exposure, unlike the continual exposure that fish would most encounter in the LCR.
- Visceral fat coverage in experimental fish was 75-100% as compared to the 25% or less in field samples. Food was not a limited resource in the experimental fish whereas it could be in the wild.
- No predatory or seasonal stress in experimental fish
- Therefore it is reasonable to predict that in a system with continual parasite exposure and severely limited ration, parasite effect would be more profound.

Treatment Regimes

- Niclosomide 200mg/kg or 5 g/kg food biweekly 2 doses
- Praziquantel 0.67 ppm in treatment water for for 24 h, hold for additional 48 h in clean water, (no more than 69 g fish/liter water)
- Placing fish back into river with infected copepods results in reinfected fish; no prophylactic or residual from treatment

Future Work

Experiment # 3 with continual exposure and .05% maintenance ration.

- Duration: minimum 23 weeks

Funding: USGS Southwest Initiative to R. Cole 1999-2003 640K

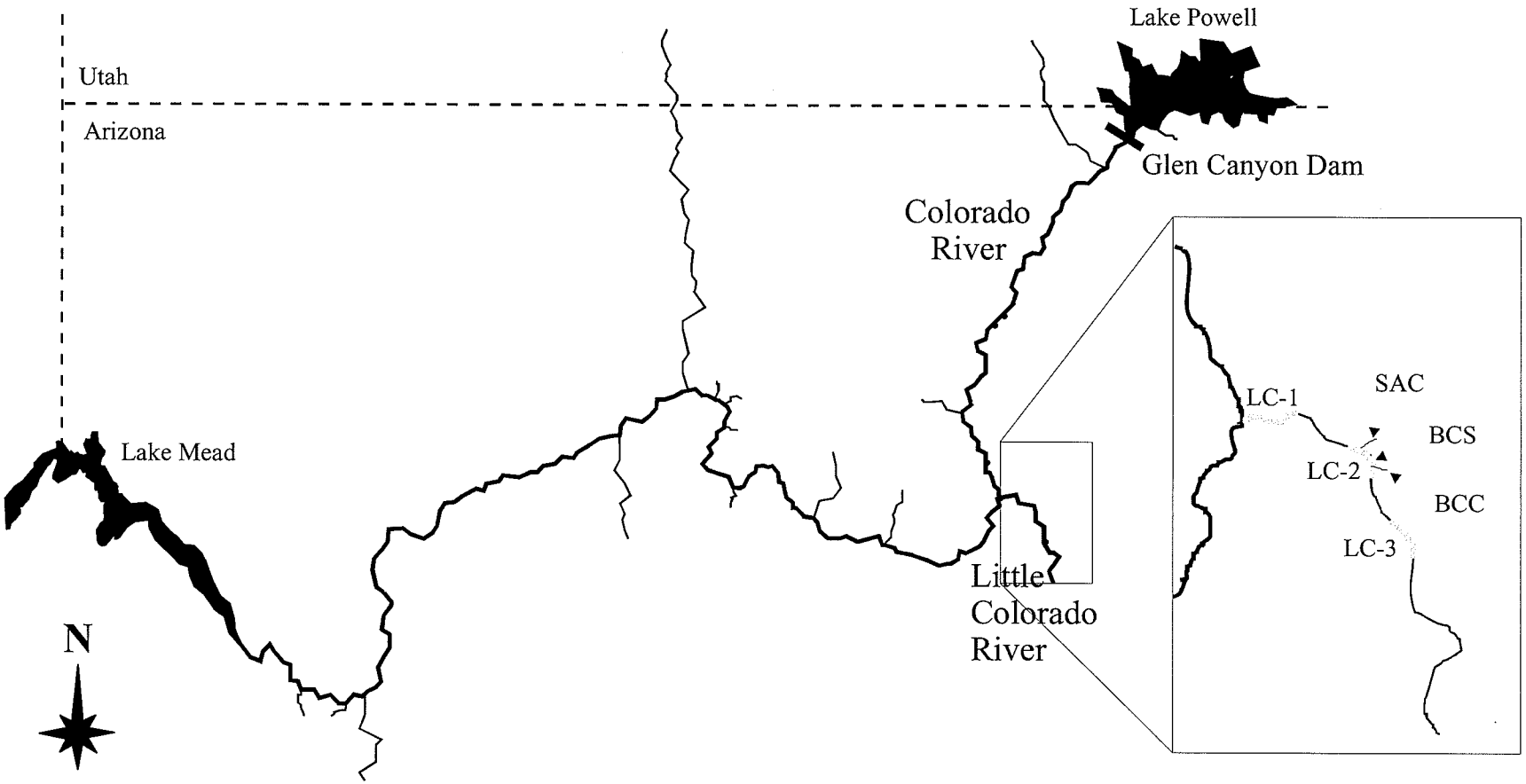
Lake Powell
Utah
Arizona
Glen Canyon Dam
Colorado
River
Lake Mead
LC-1
SAC
BCS
LC-2
BCC
LC-3
Little
Colorado
River
N

Figure 1.

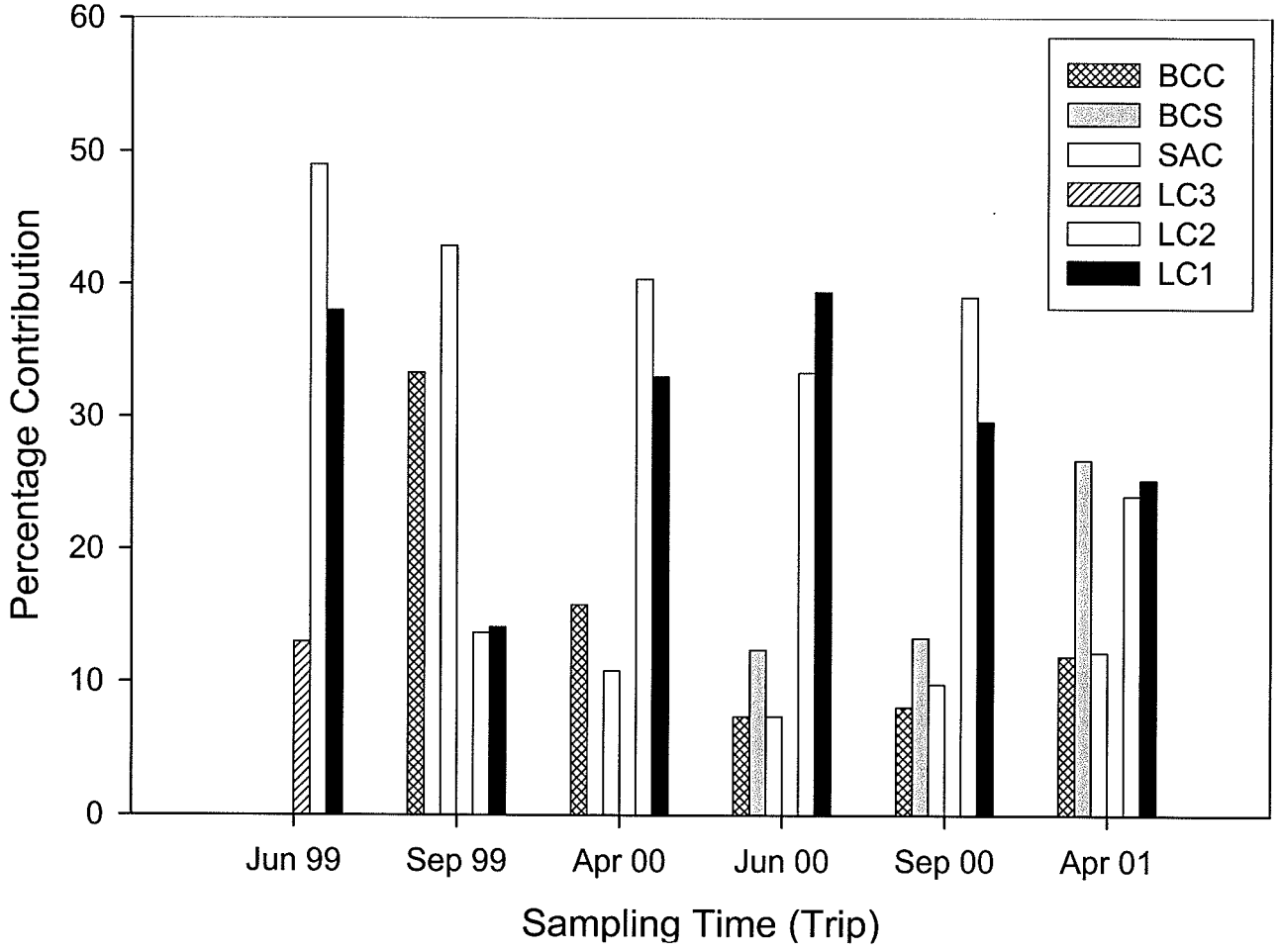
60
50
40
30
20
10
0
Percentage Contribution
BCC
BCS
SAC
LC3
LC2
LC1
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 2

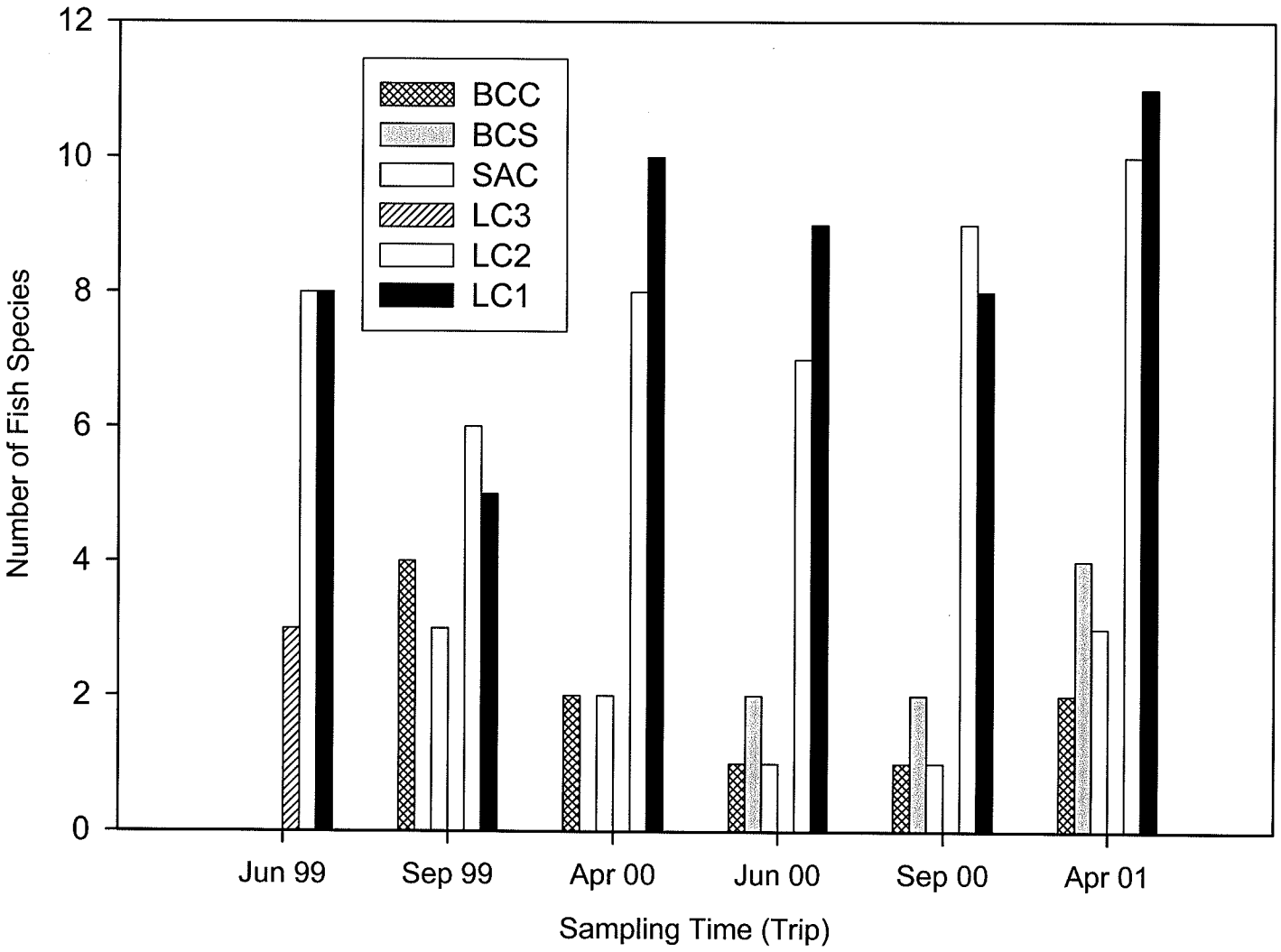
12
10
8
6
4
2
0
Number of Fish Species
BCC
BCS
SAC
LC3
LC2
LC1
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 3

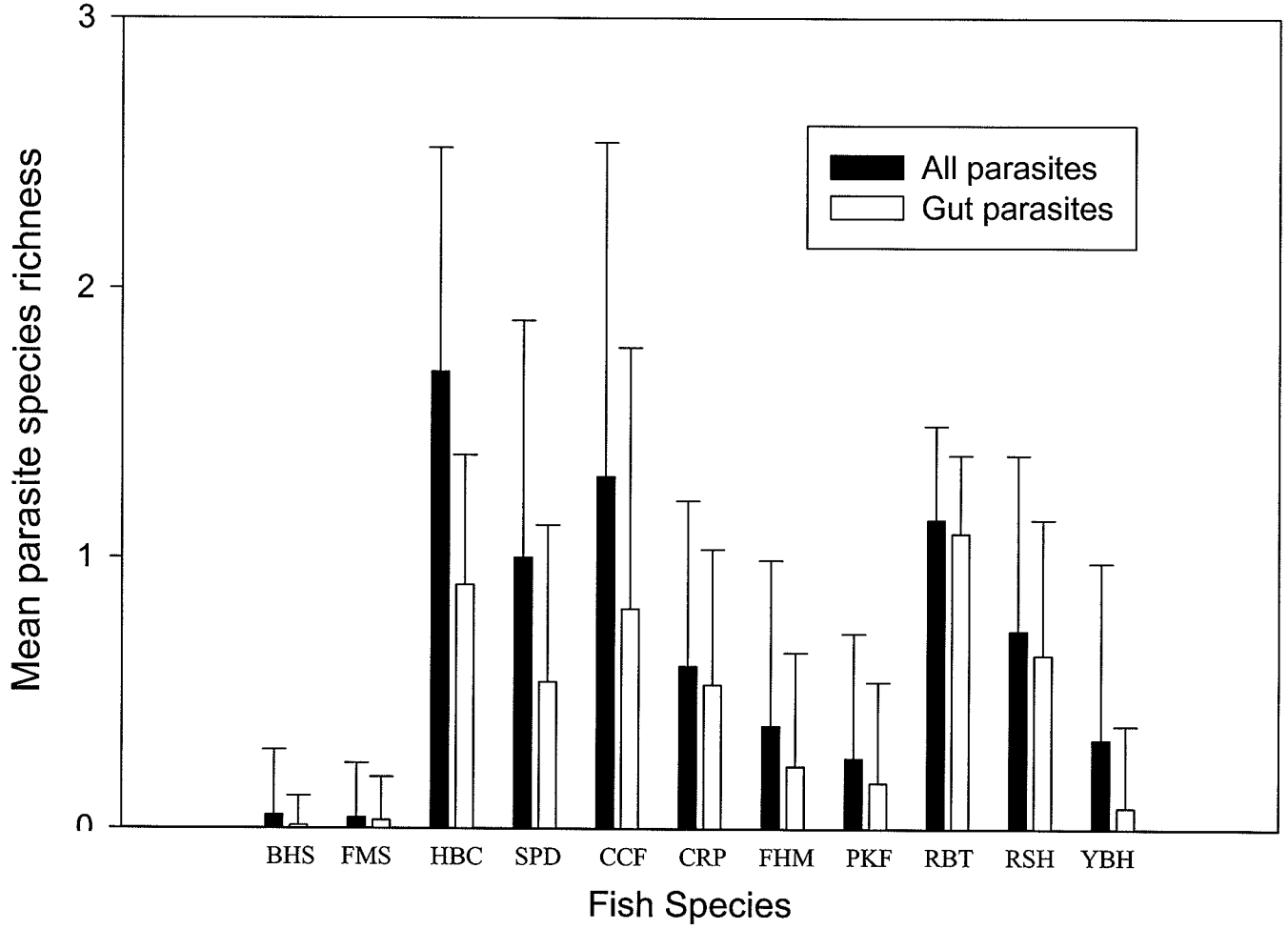
Mean parasite species richness
3
2
1
0
All parasites
Gut parasites
BHS
FMS
HBC
SPD
CCF
CRP
FHM
PKF
RBT
RSH
YBH
Fish Species

Figure 4.

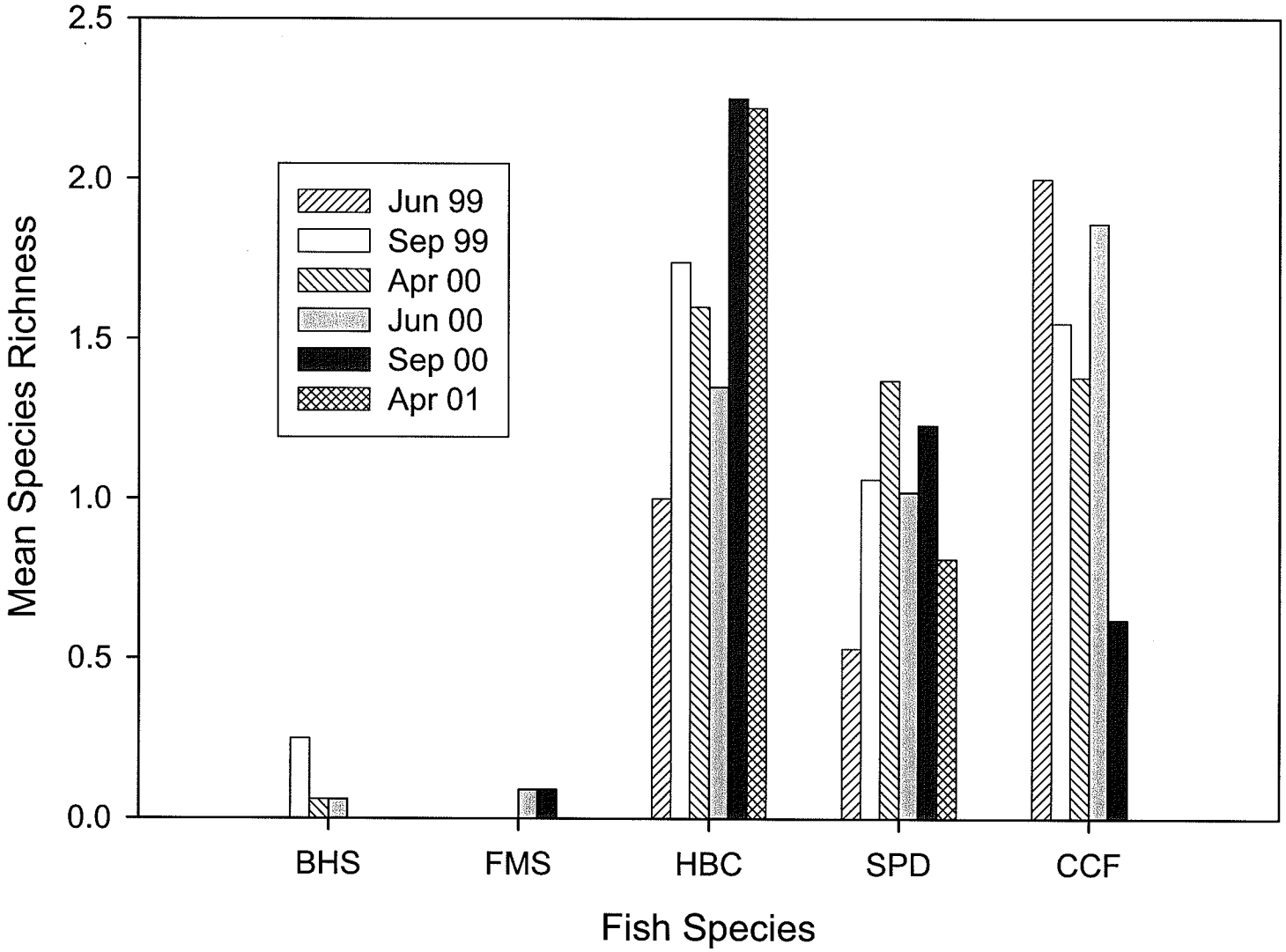
2.5
2.0
1.5
1.0
0.5
0.0
Mean Species Richness
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
BHS
FMS
HBC
SPD
CCF
Fish Species

Figure 5.

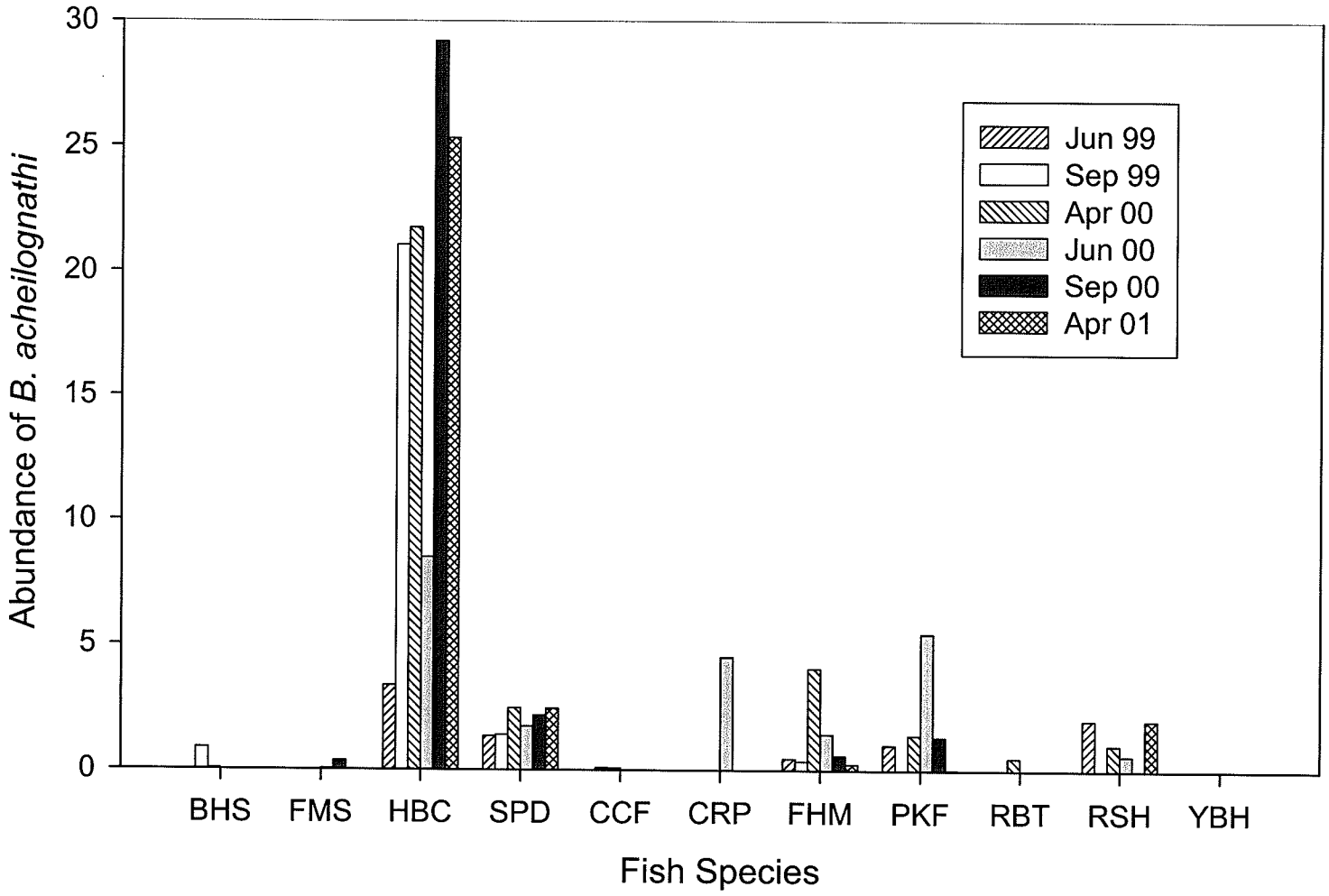
30
25
20
15
10
5
0
Abundance of B. acheilognathi
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
BHS
FMS
HBC
SPD
CCF
CRP
FHM
PKF
RBT
RSH
YBH
Fish Species

Figure 6.

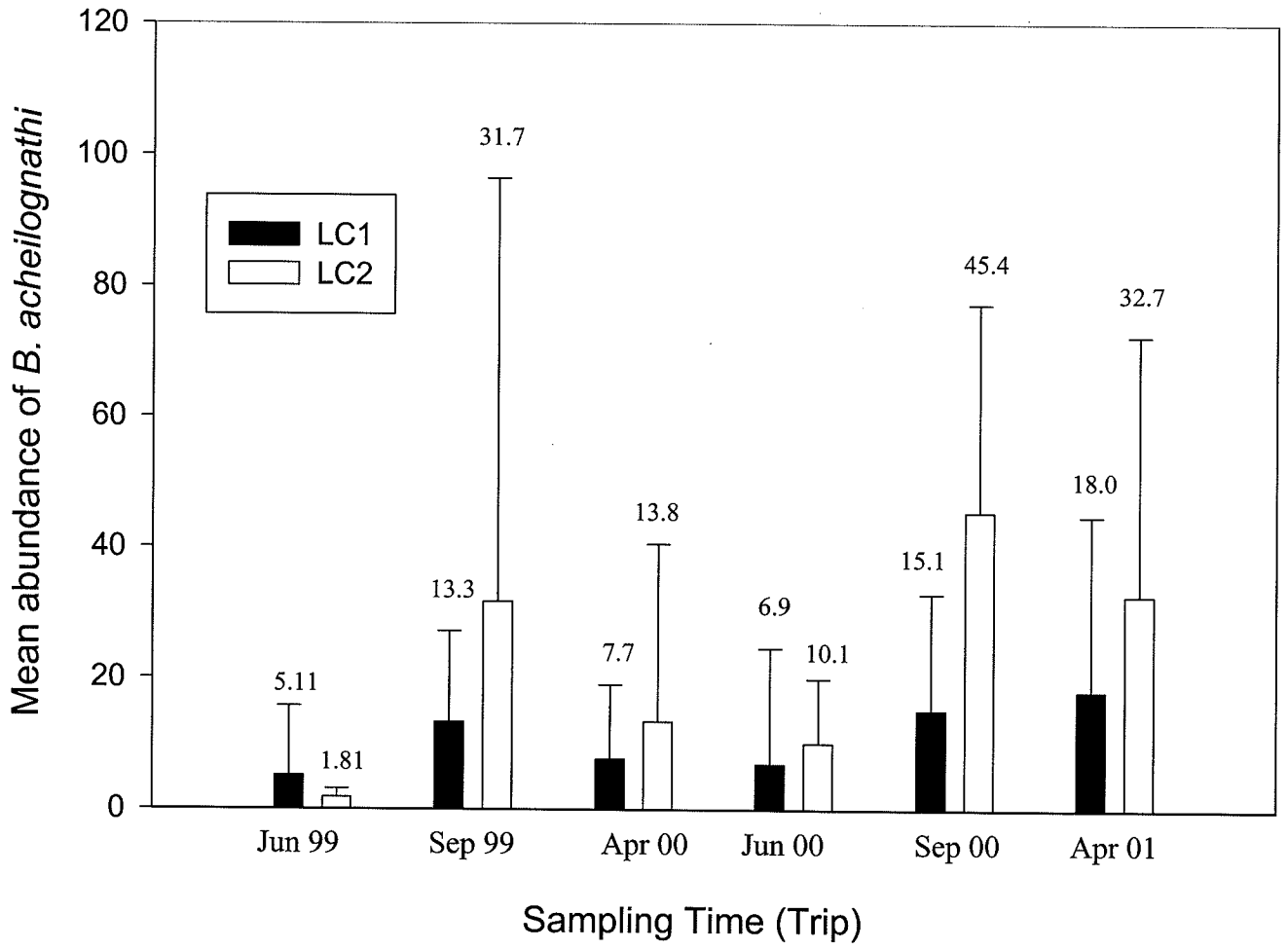
120
100
80
60
40
20
0
Mean abundance of B. acheilognathi
LC1
LC2
5.11
1.81
13.3
31.7
7.7
13.8
6.9
10.1
15.1
45.4
18.0
32.7
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 7

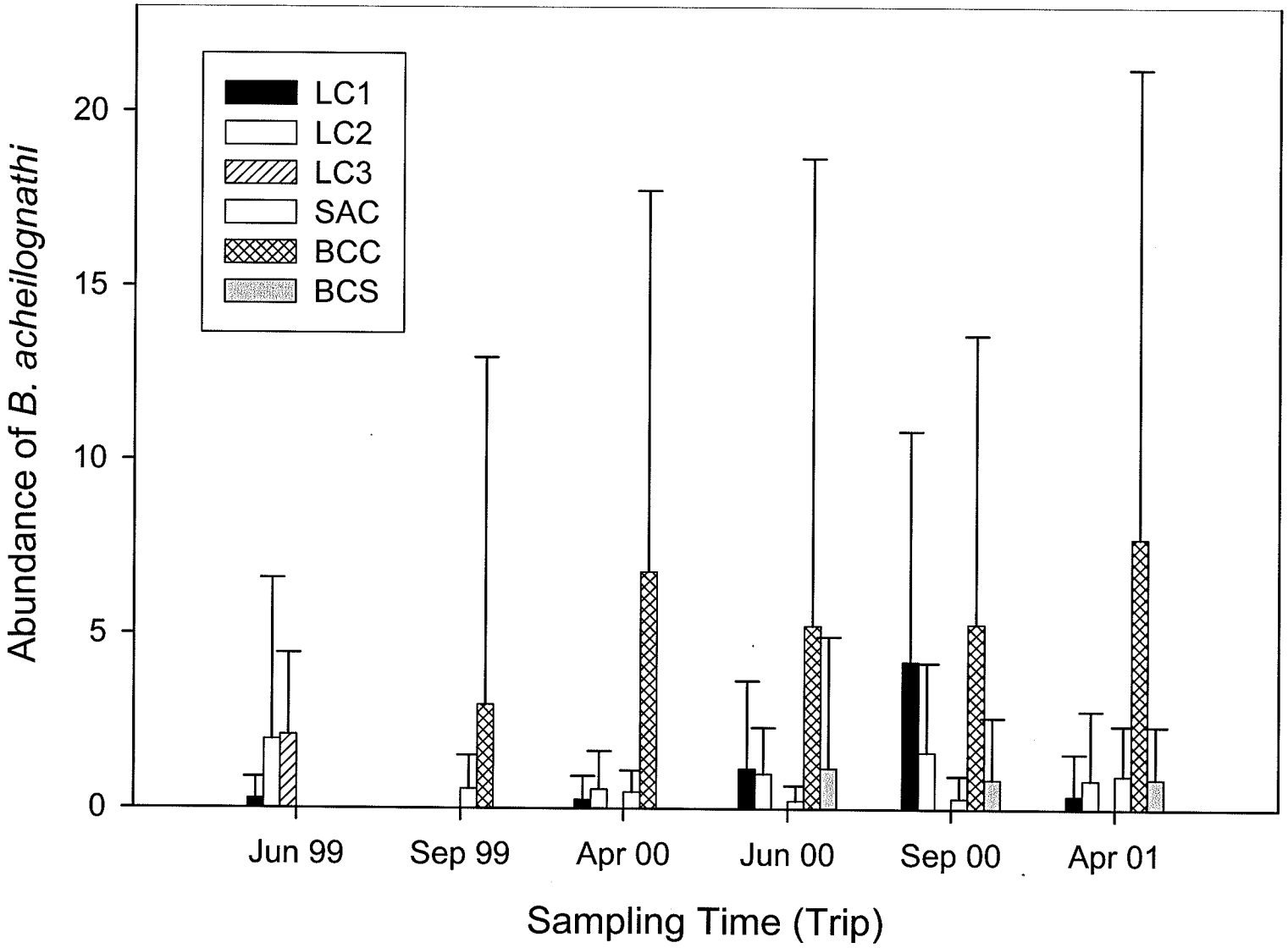
LC1
LC2
LC3
SAC
BCC
BCS
Abundance of B. acheilognathi
0
5
10
15
20
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 8

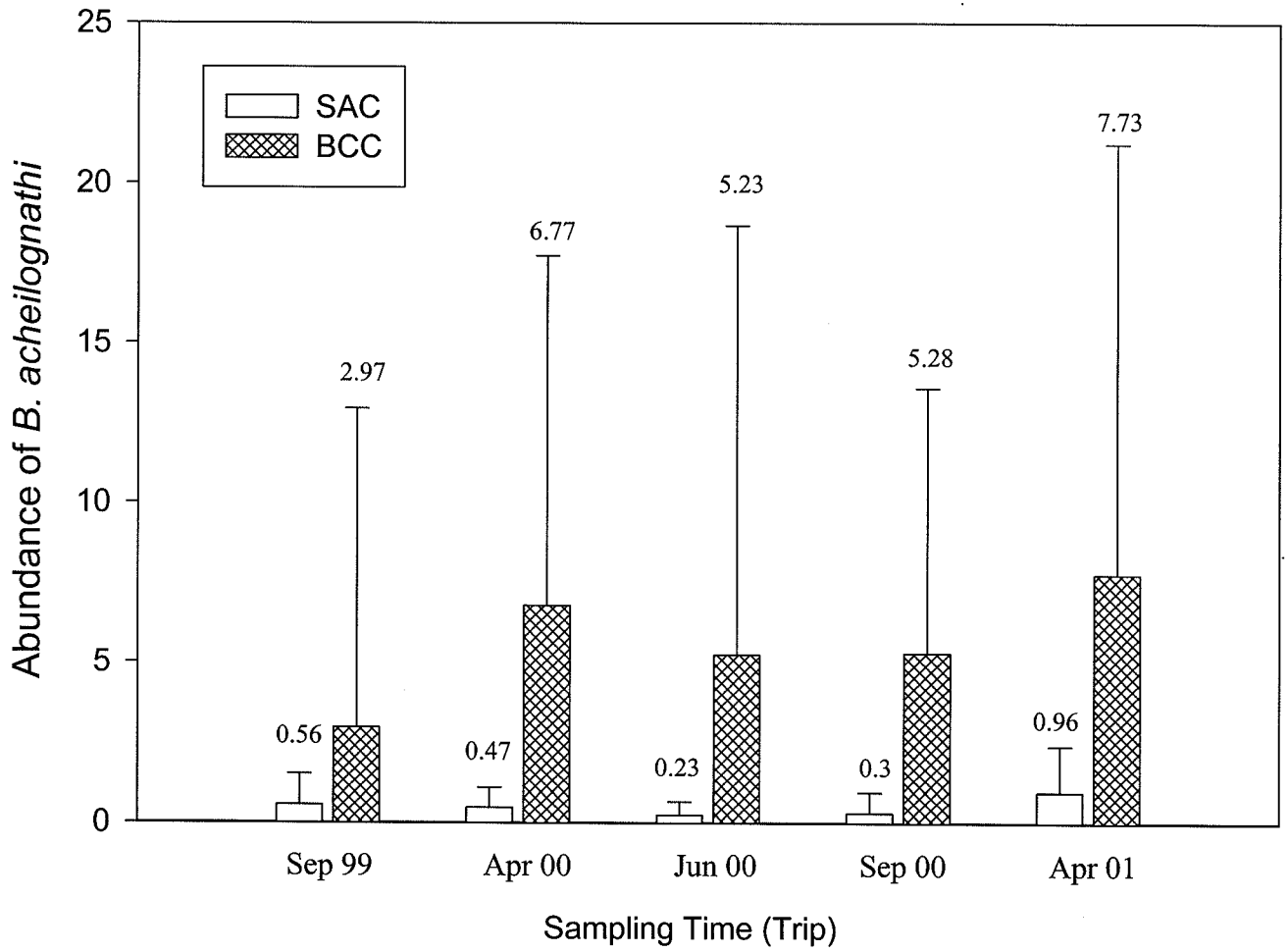
25
20
15
10
5
0
SAC
BCC
Abundance of B. acheilognathi
0.56
2.97
0.47
6.77
0.23
5.23
0.3
5.28
0.96
7.73
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 9

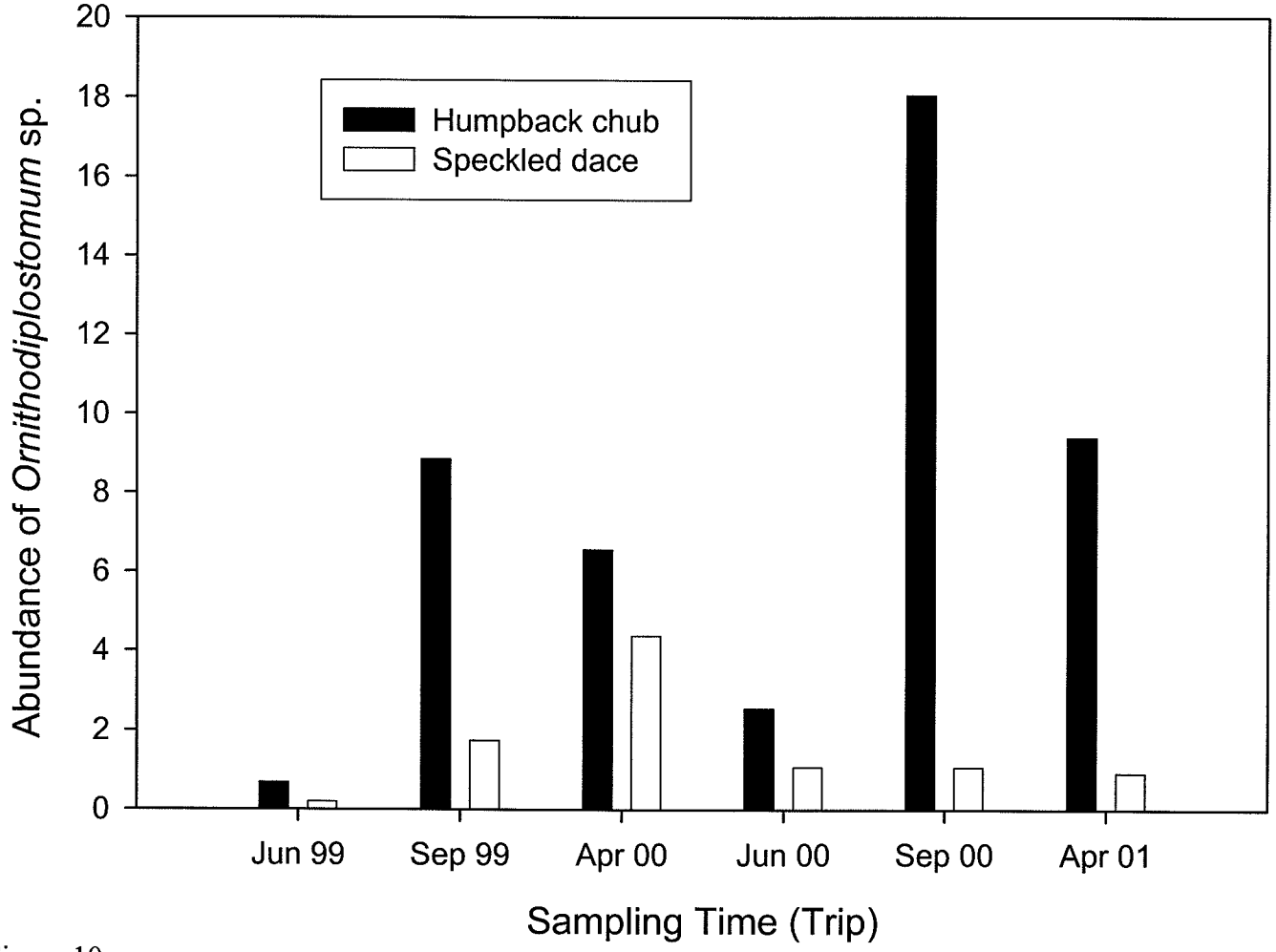
Abundance of Ornithodiplostomum sp.
20
18
16
14
12
10
8
6
4
2
0
Humpback chub
Speckled dace
Jun 99
Sep 99
Apr 00
Jun 00
Sep 00
Apr 01
Sampling Time (Trip)

Figure 10

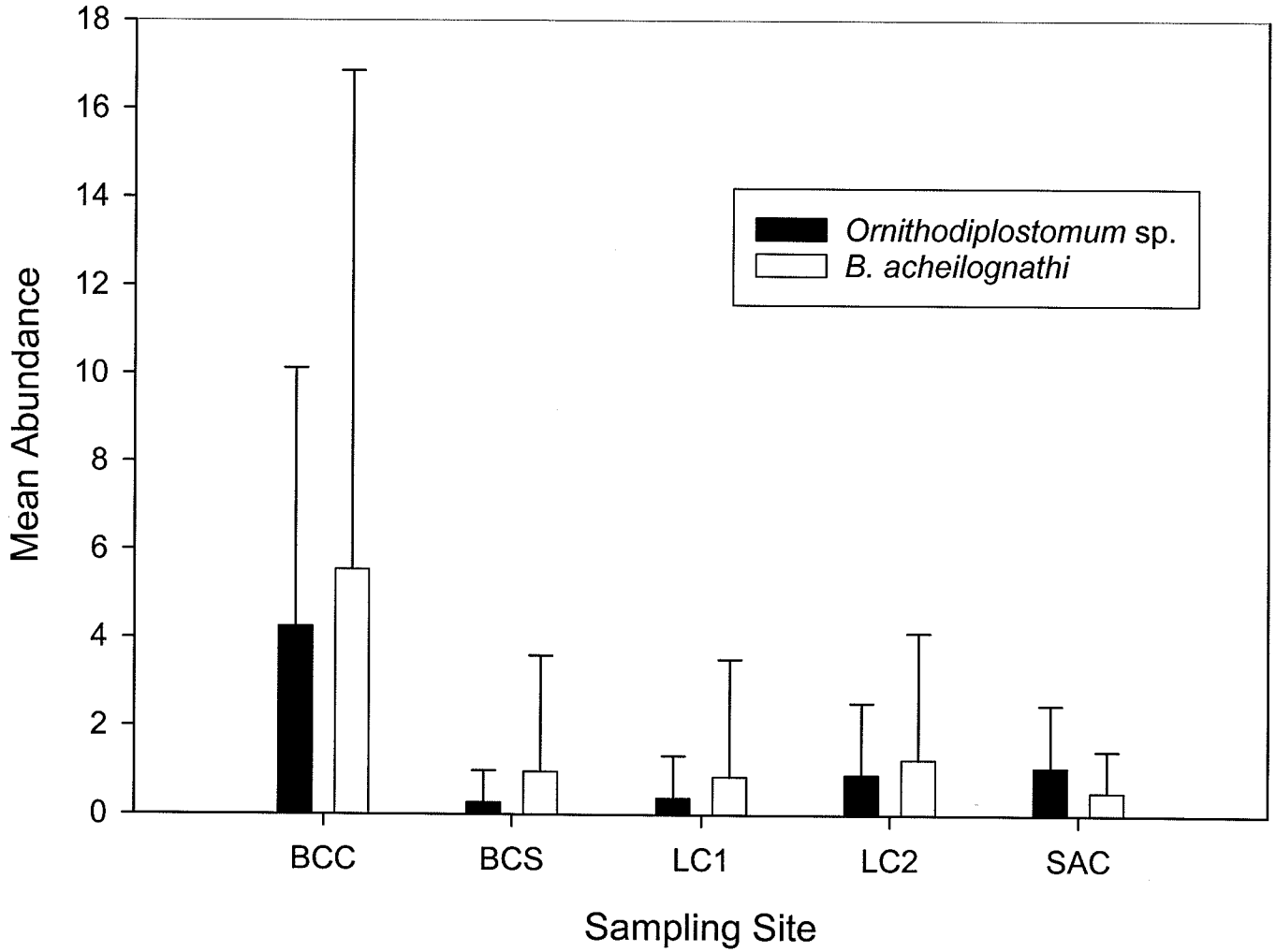
18
16
14
12
10
8
6
4
2
0
Mean Abundance
Ornithodiplostomum sp.
B. acheilognathi
BCC
BCS
LC1
LC2
SAC
Sampling Site

Figure 11

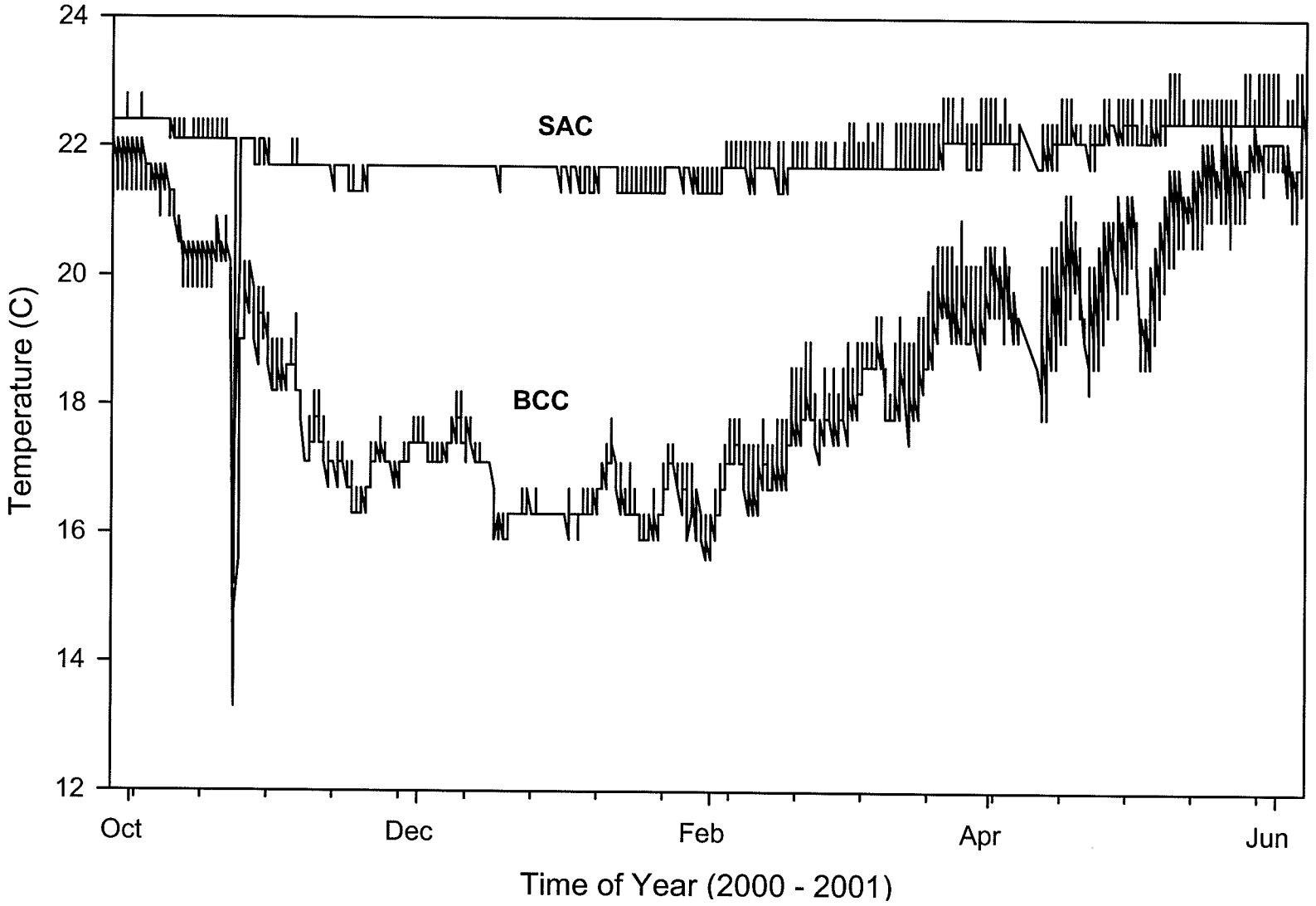
24
22
20
18
16
14
12
Temperature (C)
SAC
BCC
Oct
Dec
Feb
Apr
Jun
Time of Year (2000 - 2001)

Figure 12

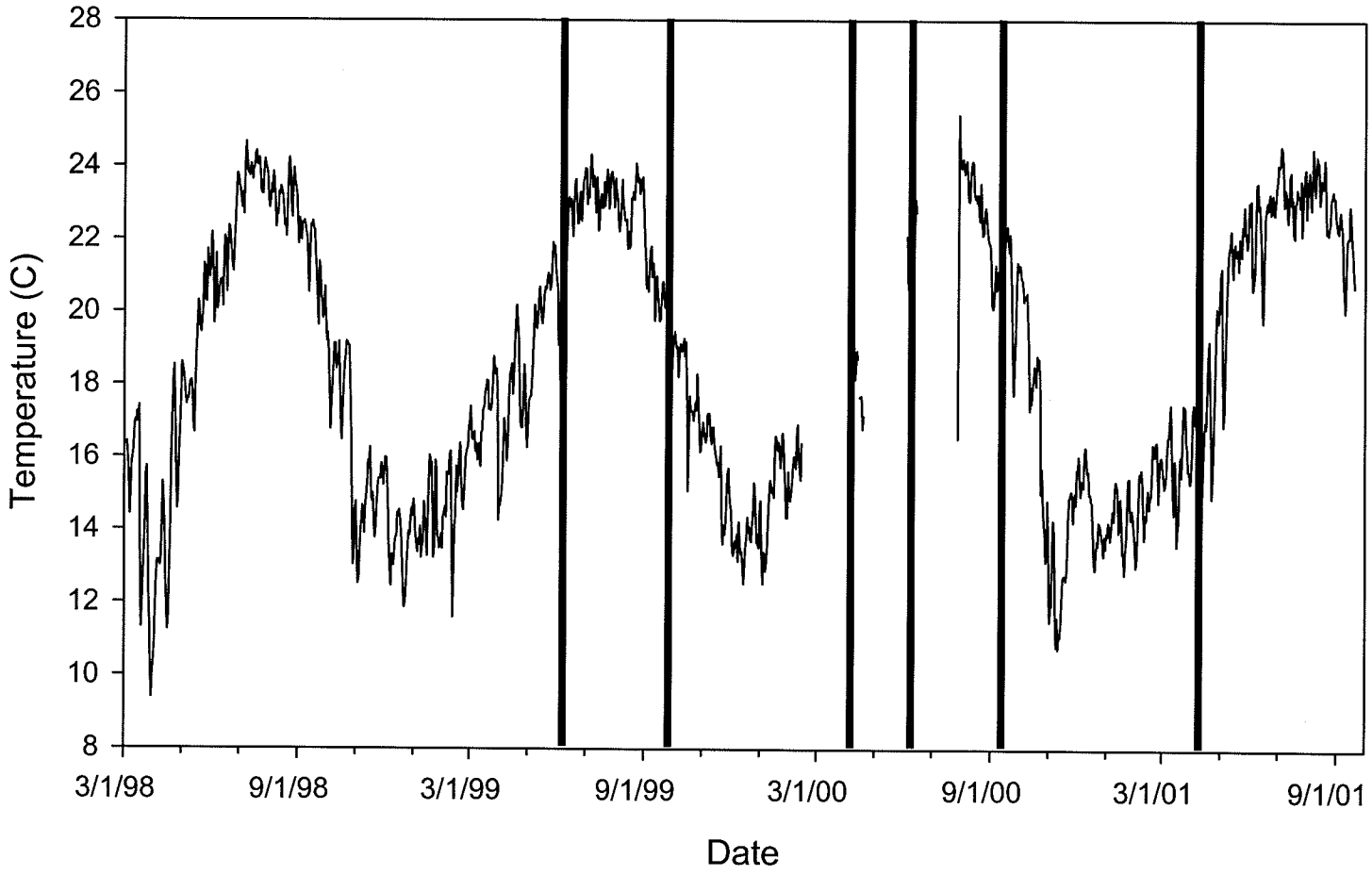
28
26
24
22
20
18
16
14
12
10
8
Temperature (C)
3/1/98
9/1/98
3/1/99
9/1/99
3/1/00
9/1/00
3/1/01
9/1/01
Date

Figure 13