

16.5 Example: New Zealand snapper (three-way case)

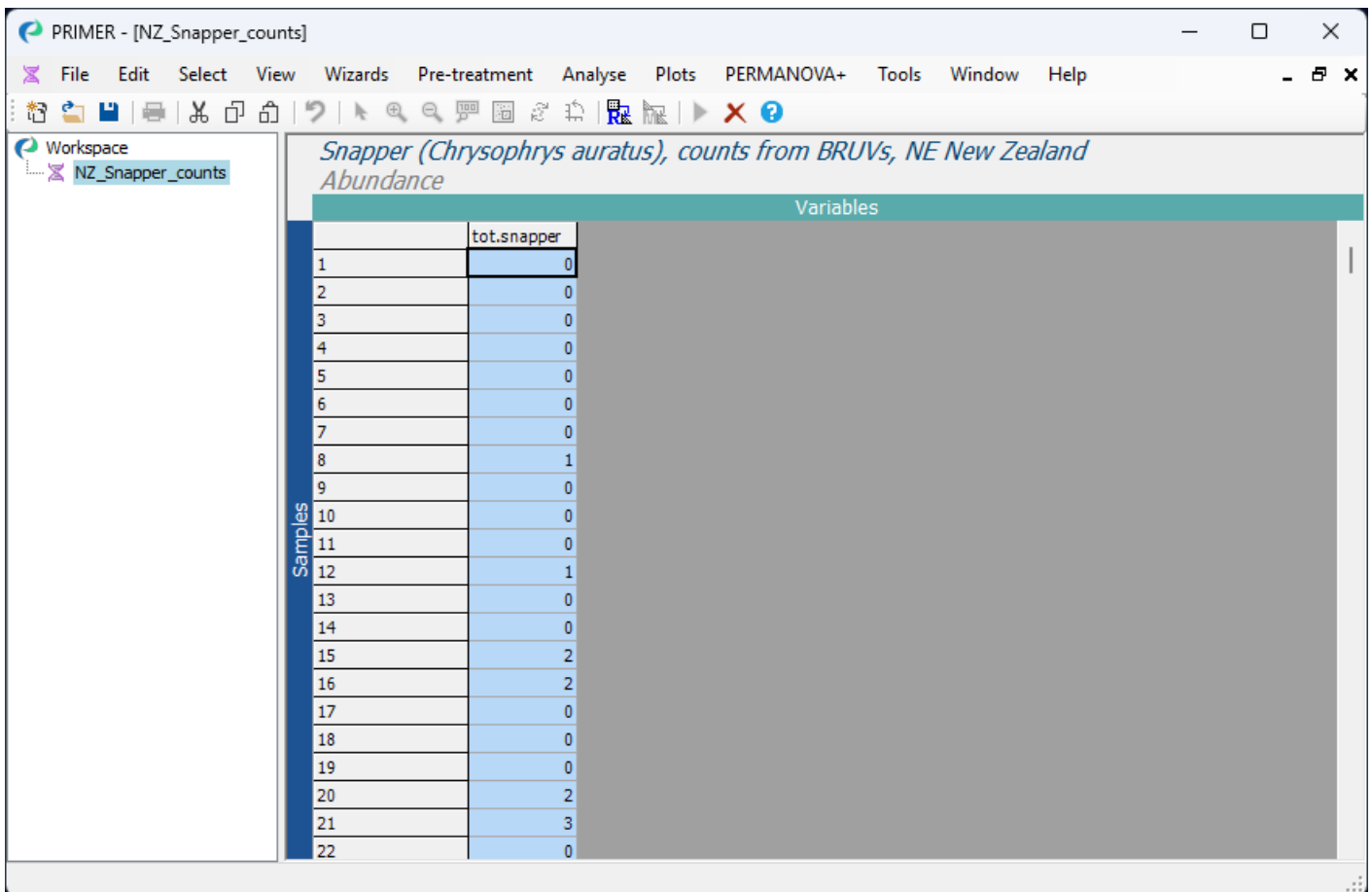
The **Means Plot** procedure in PRIMER 8 will operate just as other univariate plots do (such as histograms and dotplots) in that multiple plots will be produced - one for each variable - if there are multiple variables in the original spreadsheet. However, means plots also provide the end-user with the ability to create a multi-plot object by reference to an additional factor. This can be a second or a third factor, depending on the situation. Here, we will show a couple of examples that use this approach advantageously to show patterns across all combinations of three factors in a single multi-plot graphic.

The example dataset here are counts of snapper (*Chrysophrys auratus*) taken from baited remote underwater video (BRUV) apparatuses deployed at each of three locations: Leigh, Tawharanui and Hahei, in each of two seasons over several years (1997-2007), from sites that were located either inside or outside of a marine reserve (the factor here is 'Status', with levels 'NR' = non-reserve and 'R' = reserve). See [Smith et al. \(2014\)](#) for details.

Status by Year by Location

First, we will visualise patterns in mean relative abundances (total count per BRUV unit) of snapper inside vs outside reserves across the years at the different locations. The data are located in the file named 'NZ_Snapper_counts.pri', located in the folder 'Examples_P8' > 'NZ_Snapper_counts'.

1. Open the file 'NZ_Snapper_counts.pri' in PRIMER and click on the column (variable) name 'tot.snapper' in the sheet to highlight it, then click **Select > Highlighted**. The selected variable will be highlighted in blue, thusly:



Variables	
	tot.snapper
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	1
9	0
10	0
11	0
12	1
13	0
14	0
15	2
16	2
17	0
18	0
19	0
20	2
21	3
22	0

Next, we shall plot the mean count of total snapper observed (per BRUV) with along with the 2.5% and 97.5% percentiles of the distribution of bootstrap means from 1000 bootstrap re-samples.

2. Click **Plots > Means Plot...** and choose the following options in the 'Means plot' dialog:
 - Factor A (different symbols/colours) > **Status**
 - ☒ Split into separate groups (along the x-axis) by Factor B > **Year**, and 'untick' the box '☐ Draw group separator lines'
 - ☒ Split into separate plots by Factor C > **Location**
 - Display means as > ☒ Points
 - Join means > ☒ Across levels of Factor B (within levels of Factor A)
 - Error bars > ☒ Bootstrap percentile

as shown below:

Means Plot

Factors

Factor A (different symbols/colours)
Status

☒ Split into separate groups (along the x-axis) by Factor B
Year

☐ Draw group separator lines

☒ Split into separate plots by Factor C
Location

Display means as
☒ Points ☐ Bars

Join means
☐ Do not join means
☐ Join means
☐ Across levels of Factor A (within levels of Factor B)
☒ Across levels of Factor B (within levels of Factor A)

OK Cancel

Error bars

☐ ± 1 standard error
☐ ± 1 standard deviation

Confidence interval
CI (%): 95.0

☐ t-based
☐ Normal-based
☒ Bootstrap percentile

Bootstraps: 1000

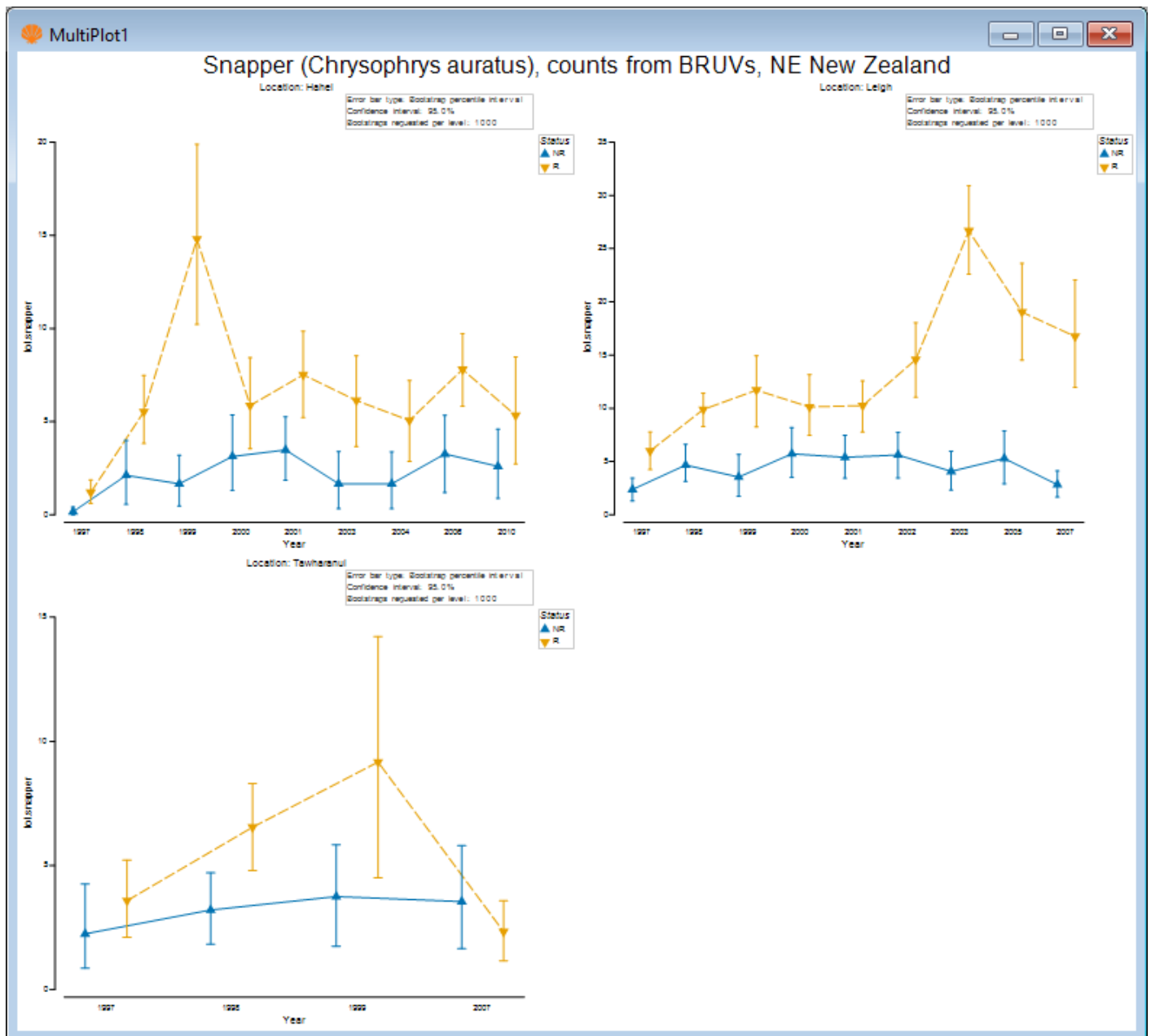
Error bars on
☒ Both sides ☐ Outer side only

Variance
☒ Separate variances ☐ Common variance

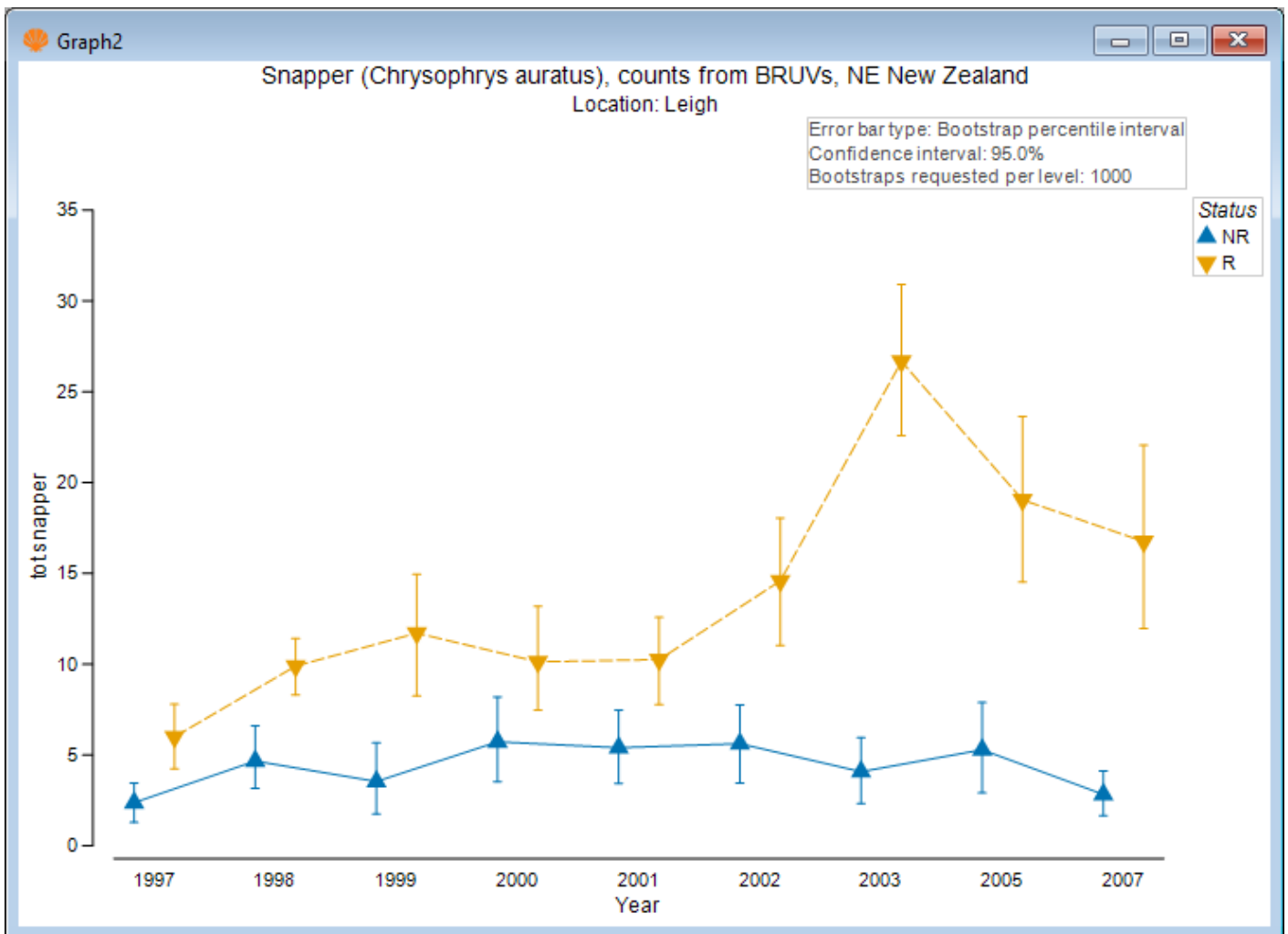
☐ Output means data to worksheet

Help

The resulting multi-plot shows the means (and bootstrap percentiles) for snapper counts from reserve and non-reserve sites separately in each year, with different locations being shown as different graphics within the multi-plot object, viz:



Examining the plot for Leigh only (click on the upper right-hand plot, or click 'Graph2' in the Explorer tree), we see, for example:



You can change or arrange the graphics in a multi-plot (i.e., by specifying the number of columns and rows of graphics that you want, and also their ordering) etc. by first clicking on the multiplot item itself in the Explorer tree (e.g., 'MultiPlot1'), then click **Graph > Special...**

Season by Status by Location

We might also like to look at differences in the mean relative abundances of snapper inside vs outside these reserves, also split by location. We will do this just for Leigh and Hahei, where we have more years of information.

- From the 'NZ_Snapper_counts' data sheet, click **Select > Samples...** then choose **Factor levels > Location** and click the 'Levels...' button. Move the words 'Hahei' and 'Leigh' from the 'Available' box on the left to the 'Include' box on the right (by clicking on them each and then clicking on the right-arrow button **>**), then click **OK**.

Select Samples

☐ Sample names
Select Sample Names...

☐ Sample numbers
1

☒ Factor levels
Location Levels...

☐ Samples that contain at least 1 non-zero value(s)

☐ Exclude samples that:

☐ Have 1 or more zero value(s)

☒ Have 1 or more missing value(s)

☐ Have 10 % or more missing values

☐ Output selection to new worksheet

OK Cancel Help

Selection

Select factor levels

Available:
Tawharanui

Include:
Hahei
Leigh

> < >> <<

Filter: Filter:

OK Cancel Help

5. Click **Plots > Means Plot...** and choose the following options in the 'Means plot' dialog:

- Factor A (different symbols/colours) > **Season**
- ☒ Split into separate groups (along the x-axis) by Factor B > **Status**, and ☒ Draw group separator lines
- ☒ Split into separate plots by Factor C > **Location**
- Display means as > ☒ Bars
- Join means > ☒ Across levels of Factor B (within levels of Factor A)
- Error bars > ☒ Bootstrap percentile

as shown below:

Means Plot

Factors

Factor A (different symbols/colours)
 Season

☒ Split into separate groups (along the x-axis) by Factor B
 Status

☐ Draw group separator lines

☒ Split into separate plots by Factor C
 Location

Display means as

☐ Points ☒ Bars

Join means

☐ Do not join means

☐ Join means

☐ Across levels of Factor A (within levels of Factor B)

☒ Across levels of Factor B (within levels of Factor A)

Error bars

☐ ± 1 standard error

☐ ± 1 standard deviation

Confidence interval

CI (%): 95.0

☐ t-based

☐ Normal-based

☒ Bootstrap percentile

Bootstraps: 1000

Error bars on

☒ Both sides ☐ Outer side only

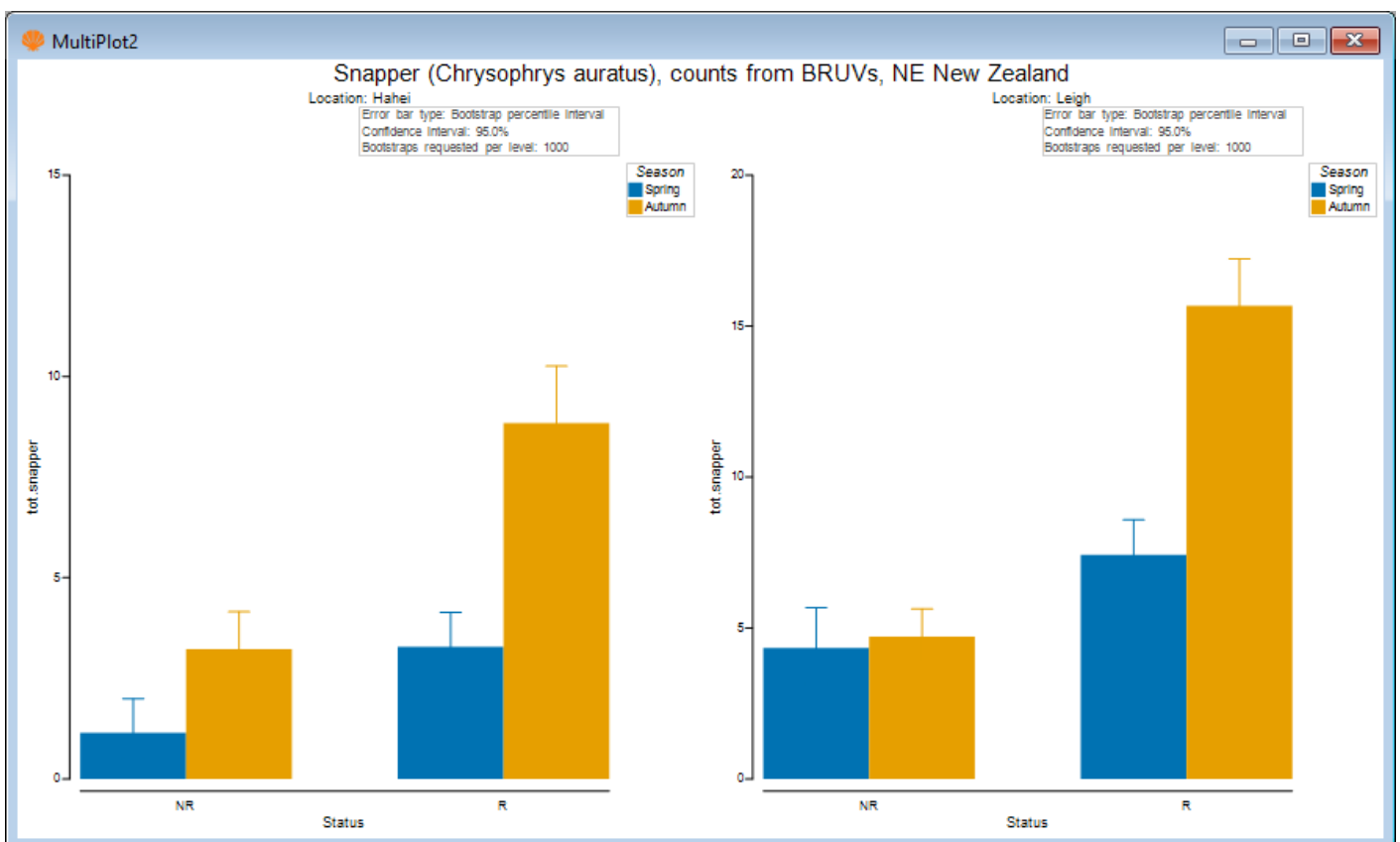
Variance

☒ Separate variances ☐ Common variance

☐ Output means data to worksheet

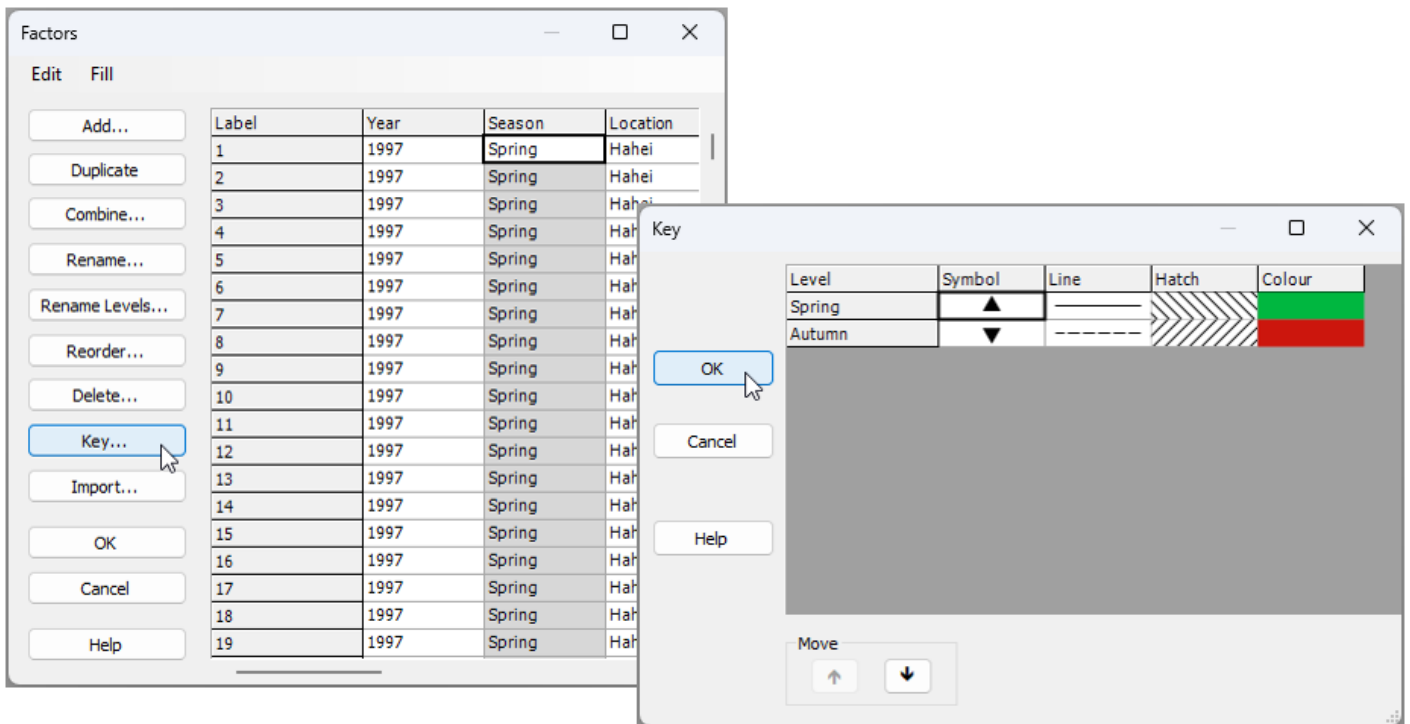
OK Cancel Help

The resulting graphic, by default, looks like this:

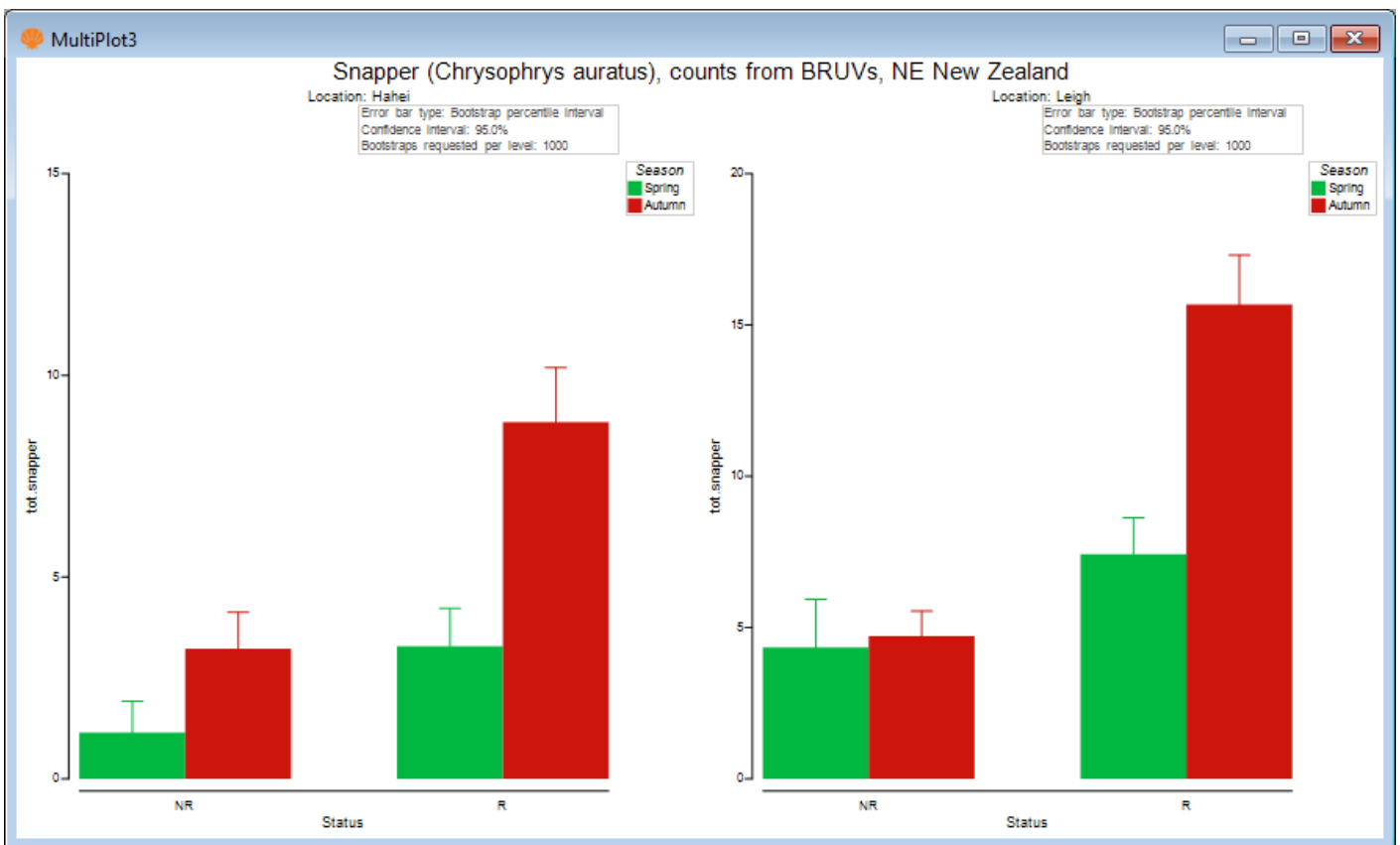


- We might like to change the colours of these bars to something a bit more reflective of spring and autumn. Go back to the original data sheet called 'NZ_Snapper_counts' and click **Edit > Factors...**, then click on the column called 'Season', and click the 'Key'

button Key.... In the 'Key' dialog, click on the colour box for 'Spring' and choose (say) a green colour, then click on the colour box for 'Autumn' and choose (say) an autumnal colour of brown or orange/red, then click 'OK'.



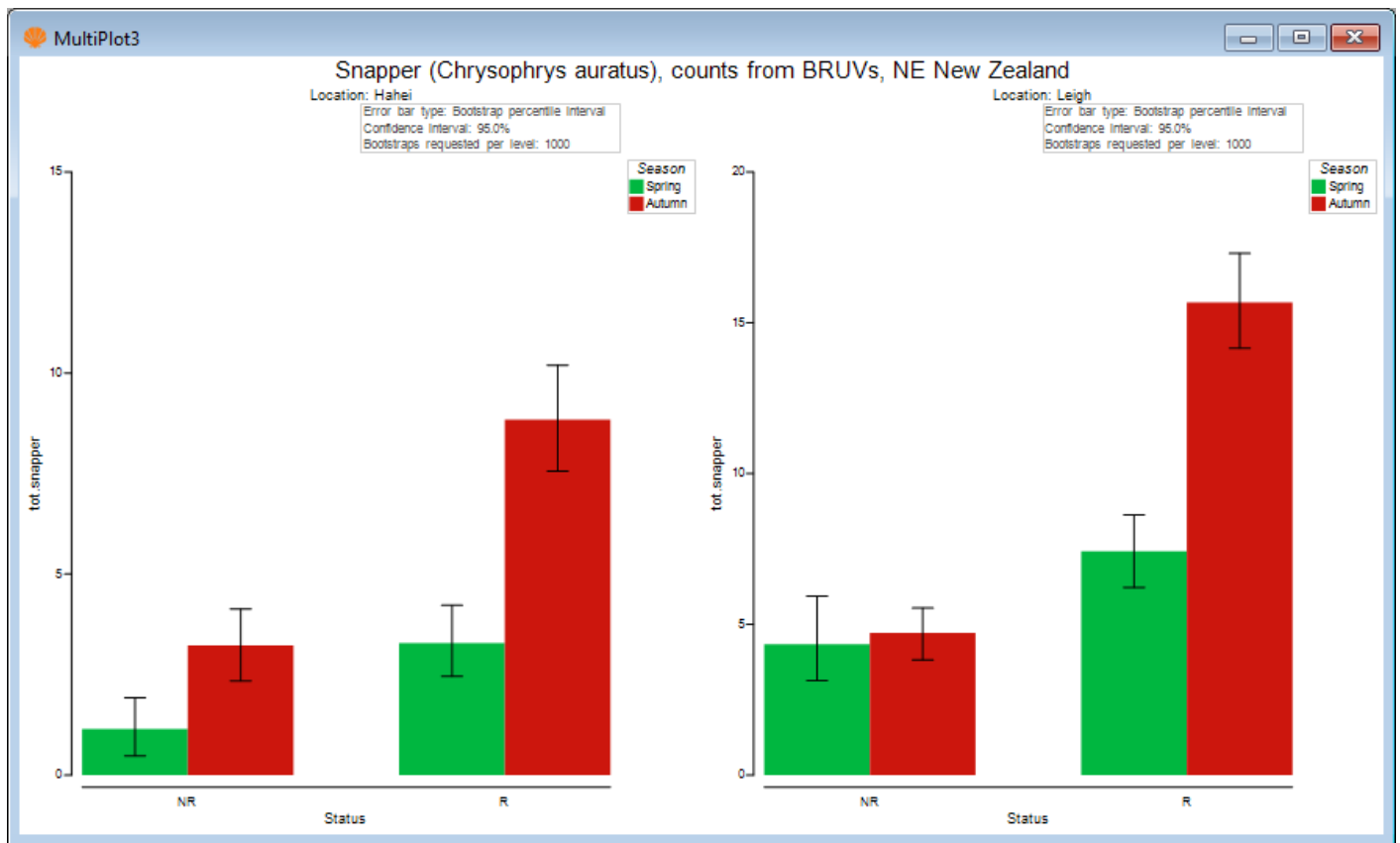
Re-running the same dialog for the means plot now as we ran before (see step 5 above), we obtain the following ('MultiPlot3'):



7. We might also like to change the error bar colours so that they are black, making it easier to see the lower and upper bootstrap percentile values. For each of 'Graph6' and 'Graph7',

in turn (both of these graphics are in 'MultiPlot3'), click **Graph > Special...**, then choose Error bars > Appearance > $\bullet$ Custom (and leave the default colour of black here).

The resulting plot looks like this:



The above plot shows that the mean relative abundance of snapper (sampled using BRUVs) is typically greater in the autumn than in the spring, on average. Also, the largest seasonal difference in mean was observed inside the reserve at Leigh.

Means plots to accompany PERMANOVA formal analysis

Typically, we would perform a more complete univariate statistical analysis of these data using (say) PERMANOVA in accordance with the full multi-factor sampling design. This could be done easily in PRIMER with PERMANOVA+ by calculating Euclidean distances among the samples based on the single variable of 'tot.snapper' and creating an appropriate Design file corresponding to the full multi-factor experimental design (including the nested factor of 'Areas' as well). Pair-wise comparisons could then be run to follow up any significant interactions that might be discovered among the main factors of interest.

It is useful to show relevant multi-factor means plots to accompany PERMANOVA tests and pair-wise comparisons. For example, a statistically significant three-way interaction of Season $\times$ Status $\times$ Location might well be accompanied by not only the relevant pair-wise comparisons, but also the above means plots that permit one to compare the means (and their variability) visually in a useful way.

Output means data to worksheet

There are many ways to customise means plots within PRIMER. It is also possible (in the 'Means Plot' dialog) to tick the box to '☒Output means data to worksheet'. This will output the means and standard errors (or the means and the upper and lower bounds of error bars of your choice) which are calculated to produce the plot. If there are customisations that you want to create in a different graphics package, or if you would rather get the values for the means (and their measures of variation) in a tabled format (e.g., for publication in supplementary material, etc.), then this extra tool is very handy, and may well be more efficient than using **Tools >**

Summary Stats..., even though the latter has been [expanded considerably](#) from what was available in version 7.

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